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RESEARCH**

APPLICATION NUMBER:

208255Orig1s000

CLINICAL MICROBIOLOGY/VIROLOGY
REVIEW(S)

VIROLOGY REVIEW
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
NDA 208255 SDN 003; Review Completed 12/16/16

Reviewer: LALJI MISHRA, Ph.D.
Date Submitted: 03/31/16
Date Received: 03/31/16
Date Assigned: 03/31/16
Sponsor: Mylan Pharmaceuticals, Inc.

Product Name(s):

Proprietary: None

Non-proprietary: Efavirenz, Lamivudine, and Tenofovir Disoproxil Fumarate Tablets

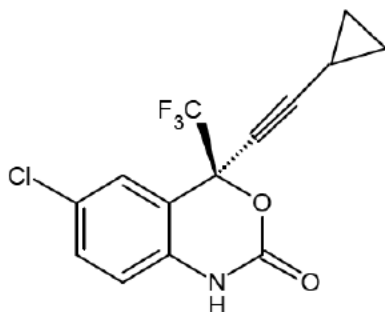
Chemical:

Efavirenz: (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one;

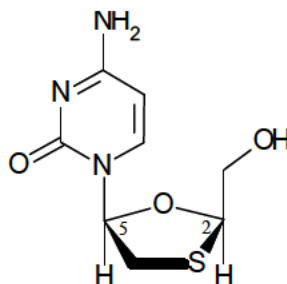
Lamivudine: (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one;

Tenofovir Disoproxil Fumarate: 9-[(R)-[[bis [[isopropoxycarbonyl)oxy] methoxyphosphino] methoxy]-propyl] adenine fumarate (1:1)

Structure:

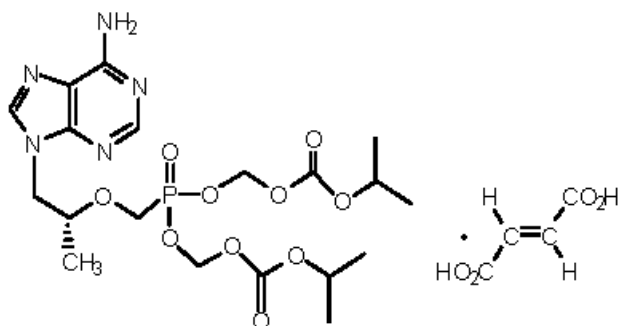


EFAVIRENZ



LAMIVUDINE

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TENOFOVIR DISOPROXIL FUMARATE

Molecular formula: C₁₄H₉ClF₃N₃O₂ C₈H₁₁N₃O₃S C₂₃H₃₄N₅O₁₄P
Molecular mass: 315.6 (b) (4) 229.26, 635 (b) (4)
Drug category: Antiviral

Indication: For the treatment of treatment of Human Immunodeficiency Virus Type 1 (HIV-1) infection in adults and in pediatric patients at least 12 years old and weighing at least (b) (4)

Dosage Form/Route of administration: Tablets/ oral

Additional submission reviewed:

Submission #	Date of correspondence	Date of Receipt	Date Assigned
NDA 208255 SDN 004	04/27/16	04/27/16	04/28/16
NDA 208255 SDN 006	09/13/16	09/13/16	09/14/16
P128512 SDN 001	12/16/15	12/16/15	01/04/16
P128512 SDN 003	04/18/16	04/18/16	04/19/16
P128512 SDN 006	05/31/16	05/31/16	06/01/16
P128512 SDN 007	07/11/16	07/11/16	07/18/16
P128512 SDN 013	12/12/16	12/12/16	12/12/16
P128512 SDN 014	12/28/16	01/05/17	01/05/17
P128512 SDN 015	01/27/17	01/27/17	01/31/17
P128512 SDN 016	02/08/17	02/08/17	02/09/17

BACKGROUND

Mylan Pharmaceuticals, Inc. has submitted a new drug application (NDA # 208255) for efavirenz, lamivudine and tenofovir disoproxil fumarate tablets under the provisions of PEPFAR (President's Emergency Plan for AIDS Relief) and under section 505(b) (2) of the Federal Food, Drug and Cosmetic Act. Mylan is seeking marketing approval of the efavirenz, lamivudine, and tenofovir disoproxil fumarate 400 mg/300 mg/300 mg combination drug tablet. The currently approved efavirenz dose in combination with other antiretroviral agents is 600 mg once daily but in this application, the sponsor is seeking approval at lower dose of efavirenz 400 mg once daily

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(b) (4) The doses for lamivudine and tenofovir disoproxil fumarate are the approved doses.

In support of the NDA 208255 the sponsor has submitted efficacy and safety data from a clinical trial, Encore1 entitled, “A randomized, double-blind, placebo-controlled, clinical trial to compare the safety and efficacy of reduced dose efavirenz (EFV) with standard dose EFV plus two nucleotide reverse transcriptase inhibitors (N(t)RTI) in antiretroviral-naïve infected individuals over 96 weeks (Evaluation of Novel Concepts in Optimization of Antiretroviral Efficacy).

The sponsor has also referred to the FDA approved NDAs for efavirenz, lamivudine and tenofovir disoproxil fumarate; NDA 020972 for Sustiva® (efavirenz) capsules (Bristol Myers Squibb Co), NDA 20564 for Epivir®(lamivudine) tablets (GlaxoSmithKline) and NDA 21356 for Viread® (tenofovir disoproxil fumarate) tablets (Gilead Sciences Inc).

NDA 20972 for Sustiva®(efavirenz) capsules received FDA approval on September 17, 1998 for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in combination with other antiretroviral agents. The recommended adult dosage for EFV is 600 mg once daily. EFV 600 mg is recommended for subjects ≥ 40 kg. Only subjects > 16 years old were eligible for the ENCORE1 study.

NDA 20564 for Epivir®(lamivudine) was approved on November 17, 1995. Epivir 300 mg once daily (OAD) dosing tablets is approved for use in combination therapy for the treatment of HIV-1 infection in adults and adolescents.

NDA 21356 for Viread® (tenofovir disoproxil fumarate 300 mg QD) tablet was approved by the FDA for the treatment of HIV-1 infection in combination with other antiretroviral agents in adults and adolescents on October 26, 2001 and for the treatment of chronic HBV infection in adults and adolescents on August 11, 2008 and August 16, 2012, respectively.

(b) (4)



In support of efficacy for efavirenz 400 mg, the sponsor has submitted results from a clinical study Encore1. The Encore1 trial was conducted by the Kirby Institute, University of New South Wales, Australia. The University of New South Wales, Australia has authorized the FDA (letter dated 01/11/16) to review their PreIND 128512 in support of the NDA 208255 filed by the Mylan Pharmaceuticals, Inc.

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The Encore1 trial is registered under ClinicalTrials.gov number: [NCT01011413](https://clinicaltrials.gov/ct2/show/study/NCT01011413) and Eudra CT (#2009-013795-44).

Comments

1. Please note that in the Encore1 trial, efavirenz 600 mg or 400 mg dose was used in combination with emtricitabine 200 mg and tenofovir disoproxil fumarate 300 mg tablets.
 (b) (4)
2. Although both emtricitabine and lamivudine exhibit overlapping resistance pathways and M184V substitution confers resistance to both emtricitabine and lamivudine, it's not known if the efficacy exhibited by lamivudine in this combination is similar to that of emtricitabine.

Please see reviews of Statistical Reviewer Wen Zeng, Ph.D. and Medical Officer Kirk Chan-Tack, M.D. for efficacy and safety analyses, respectively.

Virology data and non-nucleoside reverse transcriptase (NNRTI) and nucleoside analog reverse transcriptase (NRTI) resistance-associated substitutions identified in baseline and on-therapy isolates from subjects enrolled for the Encore1 trial are reviewed here.

Title: A randomized, double-blind, placebo-controlled, clinical trial to compare the safety and efficacy of reduced dose efavirenz (EFV) with standard dose EFV plus two nucleotide reverse transcriptase inhibitors (N(t)RTI) in antiretroviral-naïve infected individuals over 96 weeks (Evaluation of Novel Concepts in Optimization of antiretroviral Efficacy).

Primary

To compare the virological efficacy of the 2 treatment strategies as measured by the proportion of subjects with plasma HIV-1 RNA <200 copies/mL at 48 weeks after randomization. The experimental regimen of reduced dose (400 mg qd) EFV was to be judged non-inferior to the standard dose (600 mg qd) EFV if the lower limit of the 95% confidence interval for the difference in virological response between the two groups (experimental-control) did not exceed -10%. Further analyses to assess superiority were to be undertaken if non-inferiority was demonstrated in the intention to treat (ITT) and per protocol (PP) populations.

The primary objective was assessed in terms of non-inferiority at 48 weeks, with follow-up to 96 weeks.

Secondary

- To assess whether there are statistically significant differences between the two regimens in terms of other virological efficacy endpoints, immunological endpoints, safety outcomes and antiretroviral treatment change.

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- To assess whether there are statistically significant differences between the two regimens in terms of clinical events (AIDS events, deaths and serious non-AIDS events), metabolic and anthropometric changes, adherence, EFV-specific subject experience, depression anxiety stress scales (DASS) and quality of life questionnaires.

Study Design

Encore1 was an international, multicenter, randomized, two-arm, double-blind, 96-week non-inferiority trial of 600 mg once daily (qd) versus 400 mg qd EFV administered with a 2N(t)RTI backbone in antiretroviral therapy (ART)-naïve HIV-1 infected subjects. The primary analyses were conducted at 48 weeks, with subsequent follow-up and evaluation through 96 weeks. The study design is illustrated Figure 1.

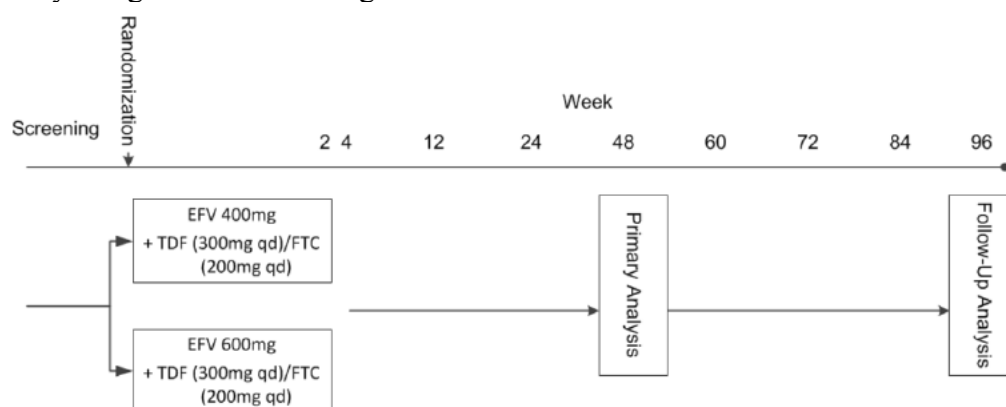


Figure 1: Study Design (Source: pre-IND 128512 SDN 000, Page 32, Figure 9.1)
EFV = efavirenz; TDF = tenofovir; FTC = emtricitabine

Microbiology Specific Inclusion Criteria

1. HIV-1 positive by licensed diagnostic test.
2. Age: >16 years of age.
3. CD4⁺ cell count between 350 and 500 cells/ μ L.
4. No prior AIDS-defining illness, using the CDC 1993 case definition (except pulmonary tuberculosis).
5. Plasma HIV-1 RNA $\geq$ 1,000 copies/mL.
6. No prior exposure to ART (including short course ARVs for preventing mother-to-child transmission [MTCT])

Microbiology Specific Exclusion Criteria

1. Active opportunistic infection or malignant disease not under adequate control.
2. Use of immunomodulators within 30 days prior to screening.

Treatments Administered

As shown in Figure 1, there were 2 treatment arms in this study as follows:

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- Arm 1 (experimental, reduced dose): EFV 400 mg qd + TDF (300 mg qd)/FTC (200 mg qd).
- Arm 2 (active comparator, standard dose): EFV 600 mg qd + TDF (300 mg qd)/FTC (200 mg qd).

In this report, the treatment groups will be referred to as EFV 400 mg (Arm 1) and EFV 600 mg (Arm 2).

Laboratory Evaluations

Table 1: Schedule of Assessments (Source; Pre-IND 128512 SDN 000 Page 43 Table 9.2]

Study Week Number ^a	SCRN ^b	0 (RAND)	2	4	12	24	36	48	60	72	84	96
Eligibility Assessments												
Informed consent	x											
Inclusion/exclusion criteria	x	x										
Medical history	x											
Pregnancy test ^c	x	x		x	x	x	x	x	x	x	x	x
Concomitant medications		x		x	x	x	x	x	x	x	x	x
Randomization		x										
Safety Assessments												
Physical examination including weight	x	x		x	x	x	x	x	x	x	x	x
Assessment of signs and symptoms		x		x	x	x	x	x	x	x	x	x
Adverse events assessment		x		x	x	x	x	x	x	x	x	x
Laboratory Tests												
Biochemistry Hematology ^{de}	x	x		x	x	x	x	x	x	x	x	x
Virology: plasma HIV-1 RNA ^f	x	x		x	x	x	x	x	x	x	x	x
Immunology T cell enumeration CD4+ CD8+ and %	x	x		x	x	x	x	x	x	x	x	x
Metabolic Assessment^g Fasting lipids and Fasting glycemics: insulin, glucose		x			x	x		x		x		x
Outcomes Assessments												
Anthropometric measures		x						x				x
Quality of life: SF-12 instrument		x						x				x
Anxiety and depression scale (DASS-21)		x						x				x
Subject experience questionnaire (SEQ)		x	x	x				x				
IRD assessment				x	x	x	x	x				
Adherence questionnaire				x				x				x
Storage samples												
Dried blood spot preparation and storage ^h		x		x	x							
Plasma preparation and serum cryopreservation ⁱ		x		x	x	x	x	x	x	x	x	x
Non-viable PBMC preparation and storage ^j		x										
Other												
Dispense study medication		x		x	x	x	x	x	x	x	x	
Pregnancy form ^k												
Genotypic resistance testing ^l												

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SCRN = screening; RAND = randomization

^a All efforts were to be made to schedule visits in keeping with the proposed week numbers. Please check the Manual for visit windows.

^b All screening assessments had to be completed within 40 days prior to the date of randomization.

^c If female and of child bearing potential.

^d Biochemistry includes: electrolytes (sodium, potassium, calcium, bicarbonate, chloride, phosphate), creatinine, albumin (if available), ALT, AST, ALK and total bilirubin.

^e Hematology includes: hemoglobin, white cells, neutrophils, lymphocytes, platelets.

^f All HIV-1 RNA assays had to be conducted at your local laboratory using locally certified HIV-1 RNA plasma viral load assays.

^g Fasting parameters include: glucose, total cholesterol, HDL cholesterol, calculated LDL cholesterol, triglycerides and insulin.

^h Dried blood spot preparation for central HIV-1 RNA assays at Week 0 and EFV concentration measurements at weeks 4 and 12.

ⁱ Central HIV-1 RNA assays on plasma. Central measurements of EFV plasma concentrations at Weeks 4 and 12. Central measurements of 25(OH)D and PTH on serum samples at Weeks 0, 12, 24, 48 & 96.

^j Non-viable PBMC was to be prepared and DNA stored for possible pharmacogenomic evaluation related to HIV disease.

^k Pregnancy form was only to be completed should a participant become pregnant on study.

^l Genotypic resistance testing should be conducted at virological failure.

Endpoints

Primary

The primary endpoint was the comparison between treatment groups of proportions of subjects with HIV-1 RNA <200 copies/mL at 48 weeks after randomization. Durability of the results was assessed over 96 weeks.

Secondary

- Proportion of subjects with plasma HIV-1 RNA <50 copies/mL and <400 copies/mL at Week 48.
- Time to achieve plasma HIV-1 RNA of <200 copies/mL (time to suppression).
- Time to virological failure (plasma HIV-1 RNA $\geq$ 200 copies/mL).
- Time to loss of virological response (TLOVR), assessed as a composite endpoint including detectable viral load ($\geq$ 200 copies/mL) following suppression, death and withdrawal or stopping randomized therapy.
- Mean changes from baseline in log plasma HIV-1 RNA copies/mL.
- Frequency of plasma HIV-1 RNA blips.

Immunological endpoints

- Mean change from baseline in CD4⁺ T cell counts/ μ L.

Time to virological failure (HIV-1 RNA $\geq$ 200 copies/mL)

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Time to virologic failure was calculated from randomization to date of first of two HIV-1 RNA ≥ 200 copies/mL in subjects who were previously suppressed (HIV-1 RNA < 200 copies/mL).

- If a subject never achieved a confirmed plasma viral load < 200 copies/mL they would be considered to have failed at Week 0.
- Missed visits were considered as failures for this analysis.

Summary

I. Disposition of Subjects

A total of 636 subjects were randomized to one of the 2 treatment arms: 324 were allocated to the EFV 400 mg dose group, 312 to EFV 600 mg dose group (Table 2). The planned recruitment of 630 subjects was achieved. After randomization, 6 subjects (3 in each treatment arm) did not start therapy or follow up. By Week 48, 5 subjects died (2 in arm 1, 3 in arm 2), 7 were lost to follow-up (4 in arm 1, 3 in arm 2), and 6 withdrew consent (3 in each arm). Six subjects (1 in arm 1, 5 in arm 2) did not complete Week 48 assessments. A total of 606 subjects (311 in arm 1, 295 in arm 2) completed the Week 48 visit.

Table 2: Subjects Disposition at Week 48 by Treatment Group (All Subjects) [Source: Pre-IND 128512 SDN 000 Page 70, Table 10.1]

	EFV 400 mg	EFV 600 mg	Total
Screened			768
Randomized	324	312	636
Received at least 1 dose of therapy and at least one follow up	321	309	630
Completed Week 48	311	295	606
Did not complete Week 48	13	17	30
Did not commence therapy or follow up	3	3	6
Died	2	3	5
Withdrew consent after starting treatment	3	3	6
Lost to follow up	4	3	7
Missing Week 48 Assessment	1	5	6

II. Baseline Characteristics

Most subjects (n = 529; 83.97%) had Category A HIV-1 disease according to the CDC category, indicating asymptomatic HIV-1 infection. The majority of subjects had acquired HIV-1 through sexual contact (n = 583; 92.5%), with a mean time since infection of about 159 weeks.

Median baseline viral load was 4.75 log₁₀ copies/mL, with 214 (33.97%) subjects having baseline viral load of $\geq 100,000$ copies/mL whereas about two-thirds (n = 416; 66.03%) had $< 100,000$ HIV RNA copies/mL. These baseline viral loads were used as stratification for the primary and secondary analyses.

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The overall mean CD4⁺ T-cell count at baseline was 272.6 ± 99.1 cells/μL and 490 (77.7%) subjects had baseline CD4⁺ T-cell counts of <350 cells/μL. Among those tested for co-infection with hepatitis B virus (HBV), hepatitis C virus (HCV) or both, 27 (4.29%) tested positive for HBV and 8 (1.27%) were HCV antibody positive.

In general, the 2 treatment groups appeared similar with respect to baseline disease characteristics.

III. Proportion of Subjects with HIV-1 RNA levels <200 copies/mL at Week 48

The results of the primary analysis are presented by treatment group and screening strata (HIV-1 RNA <100,000 vs ≥100,000 copies/mL) in Table 3.

Table 3: Proportion of Subjects with HIV-1 RNA Levels <200 copies/mL by Treatment Group and Screening Strata (Source: Pre-IND 128512 SDN 000 Page 84, Table 11.7)

Population/ Statistics	Overall			<100,000 copies/mL			≥100,000 copies/mL		
	EFV 400 mg	EFV 600 mg	Diff- erence	EFV 400 mg	EFV 600 mg	Diff- erence	EFV 400 mg	EFV 600 mg	Diff- erence
mITT Population									
N	321	309		197	197		124	112	
n	302	285		187	183		115	102	
%	94.08	92.23	1.85	94.92	92.89	2.03	92.74	91.07	1.67
Lower 95% CI	91.5	89.25	-2.1	91.86	89.31	-2.69	88.18	85.79	-5.31
Upper 95% CI	96.66	95.22	5.79	97.99	96.48	6.75	97.31	96.35	8.65
P-value	0.3578			0.3995			0.6376		
P-Int	0.8361								

All p-values from Pearson's chi-square,
P-int: p-value for interaction (Cox proportional hazards)
CI: confidence intervals

Comment

Only results for mITT population are presented in Table 3. For NC=F population and PP value, see Pre-IND 128512 SDN 000 Page 84, Table 11.7 and review of the Statistical Reviewer Wen Zeng, Ph.D.

For the primary Week 48 endpoint, the proportion of subjects with a viral load <200 copies/mL in the mITT analysis was 302/321 (94.08%) in the EFV 400 mg group and 285/309 (92.23%) in the EFV 600 mg group (difference 1.85, 95% CI -2.1 to 5.79). The lower 95% CI did not cross -10%, indicating that EFV 400 mg was non-inferior to EFV 600 mg. Using the pre-defined viral load strata at baseline (<100,000 vs ≥100,000 copies/mL), non-inferiority of the EFV 400 mg group compared to the EFV 600 mg group was also demonstrated.

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The sponsor stated (Pre-IND 128512 SDN 000 Page 85) that testing for superiority showed that the difference between the 2 treatment groups did not reach statistical significance for the mITT (p = 0.3578) population.

IV. Proportions of subjects with HIV-1 RNA Levels <50 copies/mL

The proportion of subjects with plasma HIV-1 RNA levels <50 copies/mL at Week 48 was analyzed as a secondary efficacy endpoint. The results are presented in Table 4.

Table 4: Proportion of Subjects with HIV-1 RNA Levels <50 copies/mL by Treatment Group and Screening Strata (Source: Pre-IND 128512 SDN 000 Page 86, Table 11.8)

Population/ Statistics	Overall			<100,000 copies/mL			≥100,000 copies/mL		
	EFV 400 mg	EFV 600 mg	Diff- erence	EFV 400 mg	EFV 600 mg	Diff- erence	EFV 400 mg	EFV 600 mg	Diff- erence
mITT Population									
N	321	309		197	197		124	112	
n	276	260		174	170		102	90	
%	85.98	84.14	1.84	88.32	86.29	2.03	82.26	80.36	1.9
Lower 95% CI	82.18	80.07	-3.73	83.84	81.49	-4.54	75.53	73	-8.07
Upper 95% CI	89.78	88.22	7.41	92.81	91.1	8.6	88.98	87.72	11.87
P-value	0.5172			0.5449			0.7081		
P- Int	0.897								

In the primary analysis, the lower 95% CI did not cross -10%, demonstrating non-inferiority. Using the threshold of <50 copies/mL, comparison between the 2 treatment groups for the mITT dataset showed results that were similar to that of the primary analysis, with no statistically significant difference at the 5% level between the EFV 400 mg group and the EFV 600 mg group.

Proceeding further with the secondary analysis in the mITT dataset using the viral load threshold of 400 copies/mL (Pre-IND 128512 SDN 000 Page 88, Table 11.9), similar statistically non-significant difference at the 5% level between the 2 treatment groups was observed.

Comment

At Week 96, the modified intention-to-treat analysis showed that 289 of 321(90.0%) subjects in the EFV 400 mg group and 280 of 309 (90.6%) in the EFV 600 mg group had a plasma HIV-1 viral load less than 200 copies/mL (difference -0.6% [95% CI-5.2 to 4.0]; relative risk 0.99 [95% CI 0.94 to 1.05]), which fulfilled the predetermined non-inferiority criteria ([Carey et al., 2015](#)). At the less than 50 copies/mL threshold, the modified intention-to-treat analysis showed that 277 of 321 (86.3%) patients in the EFV 400 mg group and 268 of 309 (86.7%) in the EFV 600 mg

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group achieved virological suppression (difference -0.4% [95% CI -5.8 to 4.9]), also fulfilling the non-inferiority criteria (Carey et al., 2015).

V. Mean Changes from Baseline to Week 96 in log₁₀ Plasma HIV-1 RNA Levels

Mean change from baseline to Week 96 in log₁₀ plasma HIV-1 viral load did not differ between the groups in the modified intention-to-treat analysis (EFV 400 mg -3.0 [95% CI -3.09 to -2.92] vs EFV 600 mg -2.98 [-3.07 to -2.89]; difference -0.02 [-0.14 to 0.10], p=0.74) (Figure 2) or per-protocol analysis (EFV 400 mg -3.05 [-3.14 to -2.97] EFV 600 mg -3.05 [-3.14 to -2.96]; difference 0.00 [-0.12 to 0.12], p=0.98).

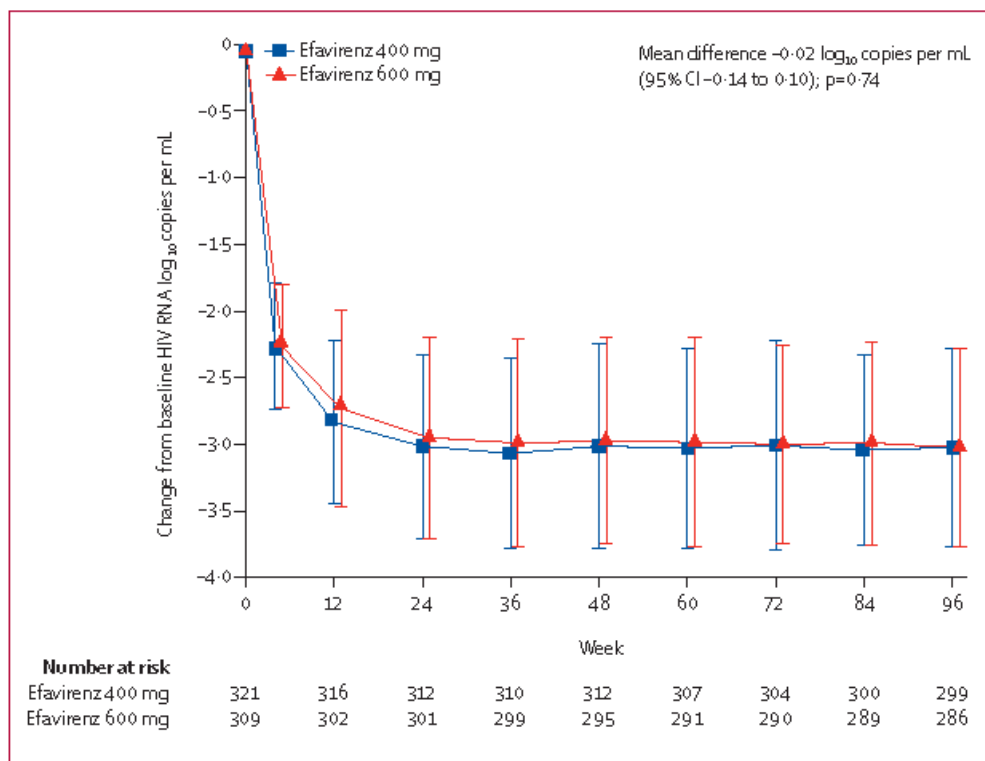


Figure 2: Mean change in HIV-1 RNA viral load from baseline to Week 96 for the modified intention-to-treat Population (Source: Carey et al., 2015).

VI. Immunological Endpoints

The immunological endpoint was the mean change from baseline to Week 96 in CD4⁺ cell count between treatment arms in the mITT (last observation carried forward) and the PP populations.

Mean CD4⁺ cell counts increased in both groups. At Week 96, mean change from baseline was 235 cells/μL (95% CI 218–252) in the EFV 400 mg group and 209 cells/μL (194–226) in the EFV 600 mg group (Figure 3). The mean change was significantly higher with the 400 mg dose of EFV in both the modified intention-to-treat population (difference 25 cells/μL [95% CI 2–48];

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p=0.03) and the per-protocol population (difference 30 cells/ μ L [5–55]; p=0.018). The mean change in CD4⁺ cell percentage did not differ between the groups (p=0.47), and neither did the mean change in CD8⁺ cell counts (p=0.74) or total lymphocyte counts (p=0.79).

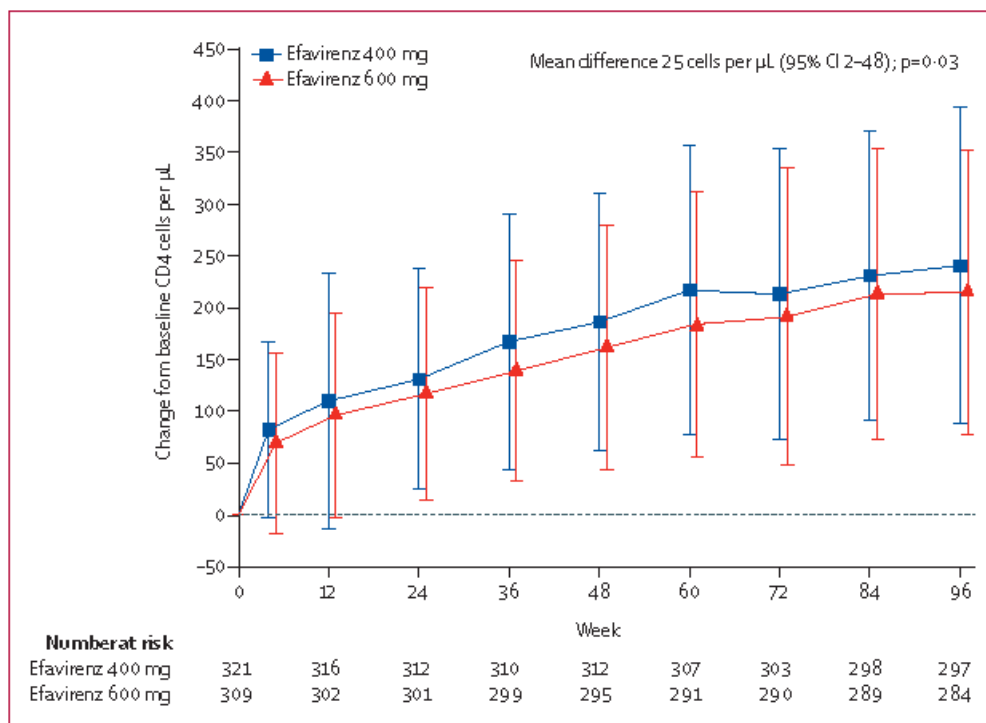


Figure 3: Mean Change in CD4⁺T-cell/ μ L from baseline to Week 96 (Source: [Carey et al., 2015](#)).

VII. Genotypic Analyses of Baseline and Treatment-Emergent Isolates from Subjects in the Encore1 Trial

Genotypic resistance data for baseline isolates were available from 286 subjects enrolled in Arm 1 (EFV 400 mg) and 282 subjects in Arm 2 (EFV 600 mg) of Encore1 trial. Phenotypic analyses of baseline and on-therapy isolates were not performed.

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Table 5: Resistance-associated Substitutions in Baseline Isolates and On-therapy Isolates from Subjects Enrolled in Arm 1 (EFV 400 mg, n=286) of Encore1 Trial (Source: IND 128512 SDN 07 Resistance data Submitted in DAVP Template Format on 07/11/16)

Subject	NNRTI Substitutions at Baseline	*NNRTI-Substitutions Emerging during Therapy	NRTI Substitutions at Baseline	*NRTI-Substitutions Emerging during Therapy	Weeks of Treatment Emerging Substitutions	Viral Load copies/mL at WK 96	
(b) (6)	A98S, K103N, Y181F	V90I , A98S, K103N, G190A	Y115N	K65R , M184V	WK60	632	
	K101E, G190A	K103N , E138A , G190A/S K103N, G190A	T69N, K70R, M184V, T215I/T, K219E	T69N, K70R, M184V, T215I, K219E T69N, K70R, M184V, T215I, K219E	WK 36 WK 72	220	
	V179E					40	
	K103N, Y181C	K103N, V179E			WK 60	986	
	V90I		A62V			40	
	V179D					40	
	L100V		D67G			40	
	K103N					40	
	V179D					40	
		V108I , K103N			M184V	WK 84	505
	K103N					40	
	V179E					40	
	V179D					40	
	K103N					40	
	V179E					40	
	K103N	K103N				WK60	114
	K103N					40	
	A98S					40	
	K103N, V106M					40	
	V106I			M41L, T215C		40	

*=Emerging substitutions shown in bold

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Table 6: Resistance-associated Substitutions in Baseline Isolates and On-therapy Isolates from Subjects Enrolled in Arm 2 (EFV 600 mg, n=282) of Encore1 Trial (Source: IND 128512 SDN 07 Resistance data Submitted in DAVP Template Format on 07/11/16)

Subject	NNRTI Substitutions at Baseline	*NNRTI-Substitutions Emerging during Therapy	NRTI Substitutions at Baseline	*NRTI-Substitutions Emerging during Therapy	Weeks of Treatment Emerging Substitutions	Viral Load copies/mL at WK 96
(b) (6)		K103N			WK 12	ND ⁺
		K101E			WK 72	3449
		K103R, V106M, V179D, F227L		M41L, K65R, M184V	WK 36	40
	V179D					50
		G190R			WK 60	40
	V108I, V179E					ND ⁺
	K103N					40
	V106I		T69N			40
	V179E	V179E			WK 60	96
	K103N					40
	V90I, K103N, P236L					40
	K103N, V106I					40
	K103N, P225H					40
				M184V	WK 48	85300
	V106M					40
	K103N	K103N, P225H		M184V	WK 48	126
	E138A, V179D					40
	V179D					40
	K103N, V106M					40
	V179D					40

*=Emerging substitutions shown in bold

ND = not done at WK 96

⁺= virologic failure at WK 48

Results presented In Table 5 and 6 are summarized as follows:

Comment

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Amino acid substitutions A98G, L100I, K101E/Q/R, K103N/S, V106A/M, V108I, V179D/E, Y181C, Y188L, G190A/S/T, P225H, F227L and M230I/L confer resistance to EFV (Stanford database). Table 5 shows that baseline isolates from 19/286 (6.7%) subjects enrolled in Arm 1 (400 mg efavirenz) of Encore1 trial harbored NNRTI resistance-associated substitutions V90I, A98S, L100V, K101E, K103N, V106I/M, V179D/E, Y181C/F and G190A. The K103N substitution was present in baseline isolates from 8/286 (2.8%) subjects enrolled in Arm 1 of the Encore1 trial. Of these, isolates from 2 subjects (# [REDACTED]^{(b) (6)}) also contained Y181C/F substitutions in addition to the substitution K103N and isolates from another subject (# [REDACTED]^{(b) (6)}) contained K103N plus V106M substitutions. The amino acid substitution V179D/E alone was present in baseline isolates from 6/286 (2.1%) subjects enrolled in Arm 1 of Encore1 Study. Additionally, isolates from 1 subject (# [REDACTED]^{(b) (6)}) contained K101E and G190A substitutions. Baseline isolates from 1 subject each harbored V90I substitution (# [REDACTED]^{(b) (6)}), A98S (# [REDACTED]^{(b) (6)}), L100V substitution (# [REDACTED]^{(b) (6)}) and V106I substitution (# [REDACTED]^{(b) (6)}). Thus, isolates from these 5 subjects (# [REDACTED]^{(b) (6)}) accounted for 1.7 % of baseline NNRTI substitutions present in subjects enrolled in Arm 1 of the Encore1 trial.

Comments

1. Three of the 19 subjects with baseline NNRTI-resistance-associated substitutions in Arm 1 of the Encore1 trial whose baseline isolates harbored either K103N plus Y181F, or K103N plus Y181F or K101E plus G190A substitutions were virologic failures at WK 48.
2. Two of 8 subjects in Arm 1 with baseline isolates containing K103N substitutions were virologic failures at WK 96.
3. None of the 6 subjects with baseline isolates containing V179D/E in Arm 1 were virologic failures at Weeks 48 and 96.
4. In the resistance datasets submitted in IND 128512 SDN 007 dated 07/11/16, the sponsor stated that NNRTI resistance-associated substitution M230L is missing for all participants. This statement suggests that either M230L was absent in baseline and treatment isolates from all subjects, or the sponsor did not search for the M230L substitution.

Table 6 shows that baseline isolates from 15/282 (5.3%) subjects enrolled in Arm 2 (EFV 600 mg) of Encore1 trial harbored NNRTI resistance-associated substitutions V90I, K103N, V106I/M, V108I, E138A, V179D/E, P225H, and P236L. K103N substitution alone or in combination with V90I, V106I/M, P225H and P236L was present in baseline isolates from 7/282 (2.5 %) subjects enrolled in Arm 2 of Encore1 trial. Of these 7 subjects, baseline isolates from 4 subjects (# [REDACTED]^{(b) (6)}) harbored additional substitutions, V90I plus P236L, V106I, P225H and V106M, respectively. Baseline isolates from 2 subjects (# [REDACTED]^{(b) (6)}; 0.7%) harbored V106I/M substitutions alone. Additionally, baseline isolates from 6/282 (2.1 %) subjects harbored amino acid substitutions V179D/E. Baseline isolates from two of these 6 subjects (# [REDACTED]^{(b) (6)}) also harbored additional substitution V108I or E138A.

Comments

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1. Two subjects (# [REDACTED]^{(b) (6)}) of the 15 subjects with baseline isolates harboring NNRTI-resistance-associated substitutions in Arm 2 were virologic failure at Week 48. However, this subject # [REDACTED]^{(b) (6)} was a virologic success at WK 96.
2. Viral load data at WK96 for subject # [REDACTED]^{(b) (6)} with baseline isolates harboring V108I plus V179E substitutions were not provided.

NNRTI-resistance-associated substitutions V90I, A98S, K103N, V108I, E138A, V179E and G190A developed during WKs 36 to 84 in on-therapy isolates from 4 subjects (# [REDACTED]^{(b) (6)} [REDACTED]^{(b) (6)} in Arm 1 of Encore1 trial (Table 5). Of these, isolates from subject # [REDACTED]^{(b) (6)} developed K103N and E138A substitutions at WK 36 and this subject was a virologic failure at Weeks 48 and 96. It should be noted that baseline isolates from subjects # [REDACTED]^{(b) (6)} and # [REDACTED]^{(b) (6)} harbored K103N substitutions in combination with Y181C/F and the K103N substitution persisted during treatment at WK60. Baseline isolates from subject # [REDACTED]^{(b) (6)} harbored NNRTI-resistance-associated substitutions K101E and G190A and the substitution G190A persisted during treatment at Weeks 36 and 72. Subjects # [REDACTED]^{(b) (6)} and # [REDACTED]^{(b) (6)} were virologic failures at Weeks 48 and 96, and, # [REDACTED]^{(b) (6)} and # [REDACTED]^{(b) (6)} were virologic failures at WK 96. The K103N substitution present in baseline isolates from subject # [REDACTED]^{(b) (6)} persisted during treatment at Week 60. However, subject # [REDACTED]^{(b) (6)} was a virologic success at WKs 48 and 96.

Nine subjects in Arm 1 of the Encore1 trial were identified as virologic failures at WK 48 (subjects who did not achieve protocol defined primary efficacy endpoint of viral load <200 copies/mL at WK48, Appendix 3). Of these, on-therapy isolates from 1 subject (# [REDACTED]^{(b) (6)}) developed NNRTI-resistance-associated substitutions during treatment at WK 36.

Ten subjects (listed in Appendix 4) were identified as virologic failures at WK 96 (subjects who did not achieve protocol defined primary efficacy endpoint of viral load <200 copies/mL at WK 96) in Arm 1 of the Encore1 trial. On-therapy isolates from 4 of these 10 (40%) virologic failure subjects (# [REDACTED]^{(b) (6)}) in Arm 1 of the Encore1 trial developed treatment-emergent NNRTI- and/or NRTI-resistance-associated substitutions. HIV-1 RNA levels for subject # [REDACTED]^{(b) (6)} were >200 copies/mL at WK 96.

On-therapy isolates from 5 subjects (# [REDACTED]^{(b) (6)}) from Arm 2 of Encore1 trial developed NNRTI resistance-associated substitutions (K101E, K103N/R, V106M, V179D, G190R, P225H and F227L) during treatment at WKs 12 to 72. Amino acid substitutions K103N/R, V106M, V179D, G190R, P225H and F227L developed in on-therapy isolates from subjects # [REDACTED]^{(b) (6)} during WKs 12 to 48.

The amino acid substitution K103N present in baseline isolates from subject # 809305 persisted in on-therapy isolates and an additional substitution P225H developed in on-therapy isolates at WK 48.

Viral load data at WK 96 were not available for subject # [REDACTED]^{(b) (6)}. Subject # [REDACTED]^{(b) (6)} was a virologic failure at WK 48. Subjects # [REDACTED]^{(b) (6)} and # [REDACTED]^{(b) (6)} were virologic successes at WK 48.

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However, subject # (b) (6) was a virologic failure at WK 48 but at WK 96, HIV-1 RNA levels for this subject were below 200 copies/mL and was considered a virologic success at Wk. 96.

Ten subjects were identified as virologic failures in Arm 2 of the Encore1 trial at WK 48 (Appendix 3). Baseline isolates from subject # (b) (6) harbored V108I plus V179E substitutions and from subject # (b) (6) a K103N substitution. On-therapy isolates from subject # (b) (6) contained NNRTI resistance-associated substitutions K103N plus P225H. Additionally, on-therapy isolates from virologic failure subject # (b) (6) developed NRTI resistance-associated substitution M184V at WK 48.

Although, on-therapy isolates from 5 subjects (# (b) (6)) from Arm 2 of the Encore1 trial developed treatment-emergent NNRTI resistance-associated substitutions, HIV-1 RNA levels for 3 subjects (# (b) (6)) were below 200 copies/mL at WK 96 and these subjects were considered virologic successes. Subject # (b) (6) was a virologic failure at WK 48 and subject # (b) (6) with treatment-emergent K101E substitution was a virologic failure at WK96.

Four subjects were identified as virologic failures at WK 96 in Arm 2 of the Encore1 trial (# (b) (6); Appendix 4, Page 32). Subject # (b) (6) and subject # (b) (6) had HIV-1 RNA levels of 3,449 copies/mL and 85,300 copies/mL at WK 96, respectively. NNRTI resistance-associated substitutions were not identified at any time points in isolates from other virologic failure subjects (# (b) (6)) in Arm 2 of the Encore1 trial.

Baseline isolates from 4 subjects in Arm 1 of Encore1 trial harbored NRTI resistance-associated substitutions M41L, A62V, D67G, T69N, K70R, M184V, T215I/C and K219Q (Table 5). The substitution M184V is associated with a high level resistance to FTC. Amino acid substitutions M41L, K70R, K219E confer zidovudine (ZDV) resistance. Amino acid substitutions D67G and T215C/I are also associated with ZDV resistance (Stanford database). Amino acid substitutions A62V and T69N are associated with multidrug resistance.

Baseline isolates from 1 subject (# (b) (6)) enrolled in Arm 2 (600 mg EFV) of the Encore1 trial harbored NRTI resistance-associated substitution T69N (Table 6).

Table 5 shows that NRTI resistance-associated substitutions K65R and M184V developed during Wks 60 to 84 in on-therapy isolates from 2 subjects (# (b) (6)) in Arm 1 of the Encore1 trial. FTC resistance-associated substitution M184V developed during treatment in on-therapy isolates from subject # (b) (6) and # (b) (6) during Wks 60 to 84. In addition, the M184V substitution present in baseline isolates from subject # (b) (6) persisted during treatment in on-therapy isolates at Wks 36 and 72. Baseline isolates from subject # (b) (6) harbored NRTI-resistance substitutions T69N, K70R, M184V, T215I and K219E and these substitutions persisted during treatment at Weeks 36 and 72. As mentioned before, subject # (b) (6) was a virologic failure at WK 48. On-therapy isolates from 1 subject in Arm 2 of the Encore1 trial (# (b) (6)) developed NRTI-resistance-associated substitutions M41L, K65R, and M184V at WK 36 and isolates from 2 additional subjects in Arm 2 of the Encore1 trial (# (b) (6))

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developed M184V substitution at WK 48 (Table 6). Thus, isolates from 3 subjects developed NRTI resistance-associated substitutions during treatment at WKs 36 and 72. Of these 3 subjects with treatment-emergent NRTI-resistance-associated substitutions, subjects # (b) (6) and # (b) (6) were virologic failures at WK 48. However, at WK 96 only subject # (b) (6) was a virologic failure. HIV-1 RNA levels for subject # (b) (6) were below 200 copies/mL and this subject was a virologic success at WK 96.

VII. Association of Polymorphism with Virologic Failure in Subjects from Arm 1 and Arm 2 of the Encore1 Trial

RT amino acids (RTP1 to RTQ334) for baseline isolates from subjects with emergent substitutions and/or virologic failure were compared with the on-therapy isolates (Encore1 Resistance Data submitted on 12/12/16. Novel substitutions associated with resistance to efavirenz in Arm 1 of the Encore1 trial (other than summarized before) was not identified. Polymorphisms at 132I/M/L, 135I/K/L/R/T, 228L/H/R and 238K/N/R/Q were examined for possible association with virologic failure.

Polymorphisms at amino acid position 132 and 228 were not observed in baseline and on-therapy isolates from virologic failure subjects in Arm 1 and Arm 2 of the Encore1 trial.

135R polymorphisms were present in baseline and on-therapy isolates at WKs 48 and 96 from a virologic failure subject # (b) (6) in Arm 1 of the Encore1 trial. Similarly, 135/K/R polymorphisms were also present in on-therapy isolates at WK 48 from another virologic failure subject # (b) (6) in Arm 1 of the Encore1 trial. In addition, 135T polymorphism was present in baseline isolate from other virologic failure subject # (b) (6) in Arm 1 of the Encore1 trial. Additionally, 135T polymorphism was present in baseline and WK 60 isolates from subject # (b) (6) and in baseline isolates from subject # (b) (6) in Arm 1 of the Encore1 trial. Both subjects (# (b) (6)) had HIV-1 RNA levels below 200 copies/mL at WK 96 and are considered virologic successes.

Polymorphisms 135L/T were observed in baseline isolates from 3 subjects (# (b) (6) and # (b) (6) in Arm 2 of the Encore trial. The 135T polymorphism persisted in WK 60 isolates from only 1 subject # (b) (6). These 3 subjects with baseline 135L/T polymorphisms had HIV-RNA levels <200 copies/mL at WK 96 and are considered virologic successes.

Polymorphism 238R was observed in baseline isolates from virologic failure subjects in Arm 1 of the Encore1 trail.

Polymorphisms 238K/R/N were not observed in baseline and on-therapy isolates from virologic failure subjects in Arm 2 of the Encore1 trial.

Table 7: Association of Polymorphism with Virologic Failure in the Encore1 Trial (Source: IND 128512 SDN 13, Resistance Datasets dated 12/12/16)

Subject	EFV Arm	Week	RT Amino Acid Position				Viral Load
			I132	I135	L228	K238	

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							copies/mL at WK 96
(b) (6)	1	0		R			869
		48		R			
		96		R			
	1	0		T		R	1076
	1	48		K/R			266
	1	0		T			114
		60		T			
	1	0		T			40
	2	0		T			40
		60		T			
	2	0		L			126
	2	0		T			40

Comments

1. For subjects # [REDACTED] (b) (6), polymorphisms for on-treatment isolates are not provided. It is assumed that RT of on-treatment isolates from these subjects contained 132I, 135I, 228L and 238K amino acids.
2. For subject # [REDACTED] (b) (6) it is assumed that RT of baseline isolates contained 132I, 135I, 228L and 238K amino acids.

VIII. Additional RT Substitutions in Baseline and Treatment Isolates from Subjects in Arm 1 and Arm 2 of the Encore1 Trial

In an attempt to identify novel substitutions that may be associated with resistance to efavirenz in isolates from subjects receiving EFV 400 mg (Arm 1) and EFV 600 mg (Arm 2) in the Encore1 trial, the sponsor was requested to provide following information.

1. Please rearrange RT resistance dataset for RT positions beginning at HIV1 reverse transcriptase amino acid position 1 in increasing numeric order, and not in alphabetic order.
2. Please generate a resistance report identifying all amino acid positions where an emergent substitution was observed in subjects treated with efavirenz 400 mg and 600 mg in the Encore1 trial.

Response

The sponsor has prepared two Excel spreadsheets that address Questions 1 and 2. Each spreadsheet contains one work book corresponding to a treatment arm in the study. The contents of each are a per patient listing of pre-randomization amino acid substitutions in RT and those detected at the time of treatment failure where a sequence was recovered. In some patients, the sponsor does not have paired samples and as such in these patients it is not possible to determine

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which substitutions are treatment emergent. The substitutions observed in any given sample are listed numerically. The tables also specify which, if any, of the observed substitutions are believed to confer any level of resistance to NNRTI and/or NRTI drugs using standard interpretations.

Comment

The sponsor submitted new resistance data in Excel sheets. Those substitutions not known to be associated with resistance to NNRTI and NRTI and marked in red font in the Excel sheets by the sponsor are shown in Table 8 and Table 9. Please note that known NNRTI and NRTI resistance-associated substitutions present in baseline and treatment isolates for subjects in Arm 1 and Arm 2 of the Encore1 trial are shown in Table 5 and Table 6 and discussed earlier in this review.

Table 8: Additional* RT Substitutions in Baseline and Treatment Isolates from Subjects in Arm 1 of the Encore1 Trial (Source: Resistance Data Excel sheet submitted on 02/08/17)

Subject ID	PID	WK	RT Substitution (Reference HXB2 sequence)
(b) (6)	2003002	WK 0	E29E/K, K32K/Q
		WK 48	D324D/E
	2101002	WK 0	Y115N/Y, D121D/E, F124F/L/S, L168F/L, K173K/N/T, S251N/S, E291D/E, V292I/K, L295L/R, E297D/E, L303L/W
		WK 60	K173T, S251N, V292I
	2103004	WK 0	V81V, A288A/G
		WK 72	K101Q, A288G
	2103001	WK 0	K275K/Q
		WK60	No new substitution
	2101007	WK 0	K49R, W88C/W, R211K/R;R284K/R
		WK 96	K49K/R, W88C
	2001012	WK 0	V21I/V
		WK 60	D320D/N
	1201017	WK 0	T39K/T
		WK 60	T39K
	2101126	WK 0	E36D/E, D324E
		WK 48	N265I/N
	1207109	WK 0	L310I/L
		WK 72	K32K/N
	1207110	WK 0	H208H/Q
		WK 48	No new substitution
0209104	WK0	I135R, V245K/R, I326V	
	WK 48	V60I, I135K/R, V245K, V276I/V	
0207014	WK 0	No new substitution	
	WK 60	No new substitution	

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*= Substitutions not previously shown to be associated with NNRTI and NRTI-resistance and shown in red font in Excel sheet received by E-mail on 01/30/17 and formally submitted on 02/08/17

Substitution shown in bold present in isolates of more than 1 subject.

Comment

None of the RT substitutions shown above are novel as these substitutions are present in baseline isolates and may not be treatment associated.

Table 9: Additional* RT Substitutions in Baseline and Treatment Isolates from Subjects in Arm 2 of the Encore1 Trial (Source: Resistance data submitted on 02/08/17; Excel sheet)

Subject ID	PID	WK	RT Substitution (Reference HXB2 sequence)
(b) (6)	2103108	WK 0	No substitution
		WK 12	No substitution
	2101103	WK 0	D177E/G, R211K/N/R/S,
		WK 72	D177E, I178M, V189I/V, R211K/R
	2001003	WK 0	T139A/T
		WK 72	No substitution
	2002104	WK 0	V179A, L193F/L, I202V, V245K/M, E248D, D250E, D324E
		WK 36	K102K/R, K103R, S105A/S. Q197K, F214L, D250D/E, D324E
	2003022	WK 0	K281K/R, D324E
		WK 60	M16M/T, K281R
	2101009	WK 0	D121D/E
		WK 60	No substitution
	0901004	WK 0	D17D/E, S68G/S, K122E/K, D123D/G/N/S, R125R/S, S162C/N/S/Y, K166K/R, E169E/K, R172K/R
		WK 36	S68G, K122E, D123S, I135R, K166R
	2003044	WK 0	No substitutions
		WK 60	E169E/Q
	2002113	WK 0	A272P, K277K/Q
		WK 36	A272S
	0209006	WK 0	T39A/S
		WK 48	T39A/T
	0207015	WK 0	V118I/V
		WK 48	No substitution
0207103	WK 0	F171Y, V317A/V	
	WK 48	I142I/T, F171F/Y, V317A	

*= Substitutions not previously shown to be associated with NNRTI and NRTI-resistance and shown in red font in Excel sheet by the sponsor.

Substitutions shown in bold present in isolates of more than 1 subject.

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Comment

None of the RT substitutions shown above are novel.

Additional comments were sent to the sponsor for clarification of data presented in Excel sheets. DAVP comments are shown in bold followed by the sponsor's response.

DAVP Comment 1

Are the amino acid substitutions shown changes with respect to a reference sequence?

Response

The sequences in this cohort were generated from plasma samples collected in EDTA whole blood tubes. Furthermore, this was performed using the Abbott ViroSeq HIV-1 genotyping system v2.0 assay. The ViroSeq HIV-1 genotyping system amplifies a 1.8 Kb region of the HIV-1 pol gene that spans the entire protease gene and approximately two-thirds of the reverse transcriptase (RT) gene. The generated sequences were analyzed using the online Stanford University HIV Drug Resistance database, the HIVdb algorithm (HIVdb). In this study the baseline sequences (week 0) were analyzed using the online HIVdb algorithm, version 6.3.1. The treatment failure analysis was performed on algorithm version 7.0.1. The Stanford University HIVdb algorithm uses the subtype B consensus sequence. This consensus sequence is derived from an alignment of subtype B sequences maintained at the Los Alamos HIV Sequence Database (hiv.lanl.gov). The consensus B sequence is therefore a commonly used reference sequence to which new sequences are compared in this program.

Comment

Adequate response

DAVP Comment 2

Are all changes with respect to the reference sequence shown? Any changes not shown need to be provided.

Response

All of the codon changes in both the baseline and treatment failure analyses are non-synonymous as supplied by the HIVdb program algorithm. The HIVdb program algorithm does not include synonymous changes.

Comment

Adequate response.

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DAVP Comment 3

What is the reference sequence?

Response

The consensus B sequence used by the Stanford HIVdb algorithm is as follows:



Comment

Adequate response

DAVP Comment 4

What does the red font indicate? These appear to indicate changes between the baseline and failure sequences but these needs to be confirmed.

Response

The red font indicates changes between the baseline and failure sequences.

Comment

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Substitutions shown in red font are present in baseline isolates and may not be treatment associated. Adequate response

DAVP Comment 5

What was the range of reverse transcriptase amino acid positions covered?

Response

The ViroSeq HIV-1 genotyping system genotypes the reverse transcriptase (RT) gene from codons 1 to 335.

Comment

Adequate response

DAVP Comment 6

Are there any amino acid positions not previously associated with efavirenz resistance at which there were 2 or more subjects with emergent substitutions?

Response

We found no amino acid positions not previously associated with efavirenz resistance at which there were 2 or more subjects with emergent substitutions

Comment

Adequate response

DAVP Comment 7

For Subject (b) (6) in the 600 spreadsheet, the NNRTI major substitutions cell value looks to be incorrect

Response

We can confirm the value of the substitution in the NNRTI major cell for participant #2103108 is correct.

Comment

Under the column heading “NNRTI major mutations” in the week 12 cell for this subject the value is “600” and is obviously incorrect.

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METHODOLOGY

Quantification of HIV-1 RNA

Plasma HIV-1 RNA quantification was undertaken at a single central laboratory using cryopreserved materials prepared during protocol mandated clinical visits and performed in a single batch at completion of the trial (at Weeks 48 and 96).

Plasma HIV RNA was quantified using the Abbott RealTime HIV-1 assay -an *in vitro* reverse transcription polymerase chain reaction (RT-PCR) assay for the quantitation of human immunodeficiency virus type 1 (HIV-1) in plasma from HIV-1 infected individuals. This commercial assay was used in accordance with the manufacturer's instructions (Appendix B – Abbott RealTime HIV-1 5160200R9).

Principles of the Procedure

- The Abbott RealTime HIV-1 assay consists of 3 reagent kits: Abbott RealTime HIV-1 Amplification Reagent Kit
- Abbott RealTime HIV-1 Control Kit
- Abbott RealTime HIV-1 Calibrator Kit

The Abbott RealTime HIV-1 assay uses RT-PCR to generate amplified product from the RNA genome of HIV-1 in clinical specimens. An RNA sequence that is unrelated to the HIV-1 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control to demonstrate that the process has proceeded correctly for each sample. The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the Abbott *m2000rt* instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott *m2000rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

The Abbott *m2000* comprises of two platforms; a) the sample preparation (sp) module and b) the reverse transcriptase (rt) modules. The Abbott *m2000sp* is used to prepare samples for the Abbott RealTime HIV-1 assay. Using the *mSample Preparation* kit, magnetic particle technology captures nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted and transferred to a 96 deep-well plate. The internal control is taken through the entire sample preparation procedure along with the calibrators, controls, and specimens. The Abbott *m2000sp* is also used to prepare the Abbott RealTime HIV-1 amplification reagent components (HIV-1 Oligonucleotide Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent).

The Abbott *m2000rt* during the read cycles of amplification allow fluorescent detection of amplification products as the HIV-1 and internal control probes anneal to their targets. The

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amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labelled oligonucleotide probes on the Abbott m2000rt instrument. The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is proportional to the log of the HIV-1 RNA concentration present in the original sample.

The upper limit of quantitation (ULLQ) for the Abbott RealTime HIV-1 assay is 10 million copies/mL, and the lower limit of quantitation is equivalent to the LOD (40 copies/mL for the 1.0 mL and 0.6 mL sample volume procedure, 75 copies/mL for the 0.5 mL sample volume procedure, and 150 copies/mL for the 0.2 mL).

Quantification of T-cell subsets

T-cell subset enumeration was performed at the routine pathology laboratory of participating investigational centers.

Genotypic assays for resistance characterizations

All genotypic drug resistance testing was performed using the ViroSeq™ HIV-1 Genotyping system that detects mutations in the RT and protease genes and provides a report indicating genetic evidence of viral resistance. It is a complete system that provides reagents for viral RNA isolation from plasma, RT-PCR, and sequencing. The entire protease gene and two-thirds of the RT gene are amplified to generate a 1.8 kb amplicon. The amplicon is used as a sequencing template for seven primers that generate an approximately 1.3 kb consensus sequence. The ViroSeq® HIV-1 Genotyping System Software assembles, edits, and identifies mutations within this 1.3 kb sequence. The software compares the consensus sequence with a known reference, HXB-2, to determine mutations present in the sample. Finally, using the online Stanford University HIV Drug Resistance Database the mutations are automatically analyzed and a drug resistance report with subtype characterization is generated.

All assays were performed in a single retrospective batch using cryopreserved materials prepared from protocol mandated clinical visits. All assays were performed in accordance with the manufacturer's instructions. Performance characteristics are listed on page 49 of the ViroSeq 3130 Manual (Appendix D-Viroseq HIV-1 genotyping v2.0).

Scoring rules and scores for the Stanford website can be located under "4. Drug Resistance Mutations (DRMs) and Sequence Interpretation" in http://hivdb.stanford.edu/DR/asi/releaseNotes/index.html#hivdb_mutationpenaltyHeader
Controls - The positive (8E5) control is a non-infectious HIV-1 virus at a concentration of 50,000 to 100,000 copies/mL. A 1:10 dilution of the positive control (5,000 to 10,000 copies/mL) was used with every run to ensure the appropriate performance of the reagents. It is designed to mimic patient samples throughout all aspects of the ViroSeq System testing procedure.

CONCLUSIONS

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The Encore1 trial was designed to compare the virological efficacy of the reduced dose of 400 mg efavirenz qd with the currently approved dose of 600 mg efavirenz qd in combination with emtricitabine and tenofovir in antiretroviral-naive HIV-1 infected subjects. The primary endpoint was the comparison between treatment groups of proportions of subjects with HIV-1 RNA <200 copies/mL at 48 weeks after randomization. A total of 636 subjects were randomized to one of the 2 treatment arms: 324 were allocated to the EFV 400 mg dose group, 312 to EFV 600 mg dose group. The sponsor stated that for the primary endpoint, the proportion of subjects with a viral load <200 copies/mL in the mITT analysis at Week 48 was 302/321 (94.08%) in the EFV 400 mg group and 285/309 (92.23%) in the EFV 600 mg group (difference 1.85, 95% CI - 2.1 to 5.79). The lower 95% CI did not cross -10%, indicating that EFV 400 mg was non-inferior to EFV 600 mg. Using the pre-defined viral load strata at baseline (<100,000 vs ≥100,000 copies/mL), non-inferiority of the EFV 400 mg group compared to the EFV 600 mg group was also demonstrated.

Genotypic resistance data for baseline isolates were available from 286 subjects enrolled in Arm 1 (EFV 400 mg) and 282 subjects in Arm 2 (EFV 600 mg) of Encore1 trial. Genotypic analysis of on-therapy isolates was performed from a limited number of subjects. Phenotypic analyses of baseline and on-therapy isolates were not performed.

Amino acid substitutions A98G, L100I, K101E/Q/R, K103N/S, V106A/M, V108I, V179D/E, Y181C, Y188L, G190A/S/T, P225H, F227L and M230I/L confer resistance to efavirenz (Stanford database). Baseline isolates from 19/286 (6.7%) subjects enrolled in arm 1 (400 mg efavirenz) of the Encore1 trial harbored NNRTI resistance-associated substitutions, V90I, A98S, L100V, K101E, K103N, V106I/M, V179D/E, Y181C/F and G190A. The K103N substitution was present in baseline isolates from 8/286 (2.8%) subjects enrolled in arm 1 of the Encore1 trial. Of these, isolates from 2 subjects (# [REDACTED]^{(b) (6)}) also contained Y181C/F substitutions in addition to the substitution K103N and isolates from another subject (# [REDACTED]^{(b) (6)}) contained K103N plus V106M substitutions. Three of the 19 subjects with baseline NNRTI-resistance-associated substitutions in Arm 1 of the Encore1 trial whose baseline isolates harbored either K103N plus Y181F, or K103N plus Y181F or K101E plus G190A substitutions were virologic failure at WK 48. Two of 8 subjects in Arm 1 with baseline isolates containing K103N substitutions were virologic failure at WK 96 and the remaining 6 subjects with baseline isolates harboring K103N substitution achieved HIV-1 RNA levels <200 copies/mL at WK 96.

The amino acid substitution V179D/E alone was present in baseline isolates from 6/286 (2.1%) subjects enrolled in arm 1 of the Encore1 trial. None of the 6 subjects with baseline isolates containing V179D/E were a virologic failure at Weeks 48 and 96.

Subjects whose baseline isolates harbored V90I, A98S, L100V, V06I alone achieved HIV-1 RNA levels <200 copies/mL at Wks 48 and 96 and were grouped as virologic successes.

NNRTI resistance-associated substitutions V90I, K103N, V108I, E138A, V179E and G190A developed during Weeks 36 to 84 in on-therapy isolates from 4 subjects (# [REDACTED]^{(b) (6)}) in Arm 1 of the Encore1 trial. Of these, on-therapy isolates from subject # [REDACTED]

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(b) (6) developed K103N and E138A substitutions at WK 36 and the K103N substitutions persisted during treatment at WK 72. It should be noted that baseline isolates from subject # (b) (6) and # (b) (6) harbored K103N substitution in combination with Y181C/F and the K103N substitution persisted during treatment at WK 60. Baseline isolates from subject # (b) (6) harbored NNRTI-resistance-associated substitutions K101E and G190A and the substitution G190A persisted during treatment at WKs 36 and 72. Subjects # (b) (6) were virologic failures at WKs 48 and 96. However, subject # (b) (6) was a virologic success at Wk 48 but virologic failure at WK 96. Subject # (b) (6) whose on-therapy isolates developed V018I and K103N substitutions at WK 84 was a virologic failure at WK 96. The K103N substitution present in baseline isolates from subject # (b) (6) persisted during treatment at Week 60 and subject # (b) (6) was a virologic success at WKs 48 and 96.

Baseline isolates from 4 subjects in Arm 1 of Encore1 trial harbored NRTI resistance-associated substitution M41L, A62V, D67G, T69N, K70R, Y115N, M184V, T215I/C and K219Q. The substitution M184V is associated with a high level resistance to emtricitabine.

NRTI resistance-associated substitutions K65R and M184V developed during WKs 60 to 84 in on-therapy isolates from 2 subjects (# (b) (6)) in Arm 1 of the Encore1 trial. FTC resistance-associated substitution M184V developed during treatment in on-therapy isolates from subject # (b) (6) during WKs 60 to 84. In addition, M184V substitution present in baseline isolates from subject # (b) (6) persisted during treatment in on-therapy isolates at Weeks 36 and 72. Baseline isolates from subject # (b) (6) harbored NRTI-resistance substitutions T69N, K70R, M184V, T215I and K219E and these substitutions persisted during treatment at Weeks 36 and 72. As mentioned before, subject # (b) (6) was a virologic failure at WKs 48 and 96.

Nine subjects in Arm 1 of the Encore1 trial were identified as virologic failures at WK 48 (subjects who did not achieve protocol defined primary efficacy endpoint of viral load <200 copies/mL at WK48, Appendix 3). Of these, on-therapy isolates from 1 subject (# (b) (6)) developed NNRTI-resistance-associated substitutions during treatment at WK 36.

Ten subjects were identified as virologic failures (subjects who did not achieve protocol defined primary efficacy endpoint of viral load <200 copies/mL at WK 96 (Appendix 4). On-therapy isolates from 4 of these 10 (40%) virologic failure subjects (# (b) (6)) in arm 1 of the Encore1 trial developed treatment-emergent NNRTI and/or NRTI resistance-associated substitutions. HIV-1 RNA levels for subject # (b) (6) and # (b) (6) were >200 copies/mL at WK 96.

Baseline isolates from 15/282 (5.3%) subjects enrolled in Arm 2 (EFV 600 mg) of the Encore1 trial harbored NNRTI resistance-associated substitutions V90I, K103N, V106I/M, V108I, E138A, V179D/E, P225H, and P236L. K103N substitution alone or in combination with V90I, V106I/M, P225H and P236L was present in baseline isolates from 7/282 (2.5 %) subjects enrolled in Arm 2 of the Encore1 trial. Of these 7 subjects, baseline isolates from 4 subjects (# (b) (6)) harbored substitutions, V90I plus P236L, V106I, P225H and V106M, respectively. Baseline isolates from 2 subjects (# (b) (6)); (0.7%) harbored V106I/M substitutions alone. Additionally, baseline isolates from 6/282 (2.1 %)

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subjects harbored amino acid substitutions V179D/E. Baseline isolates from two of these 6 subjects (# [REDACTED]^{(b) (6)}) also harbored additional substitution V108I or E138A.

Two of the 15 subjects (# [REDACTED]^{(b) (6)}) with baseline isolates harboring NNRTI-resistance-associated substitutions in Arm 2 were virologic failures at Week 48. However, subject # [REDACTED]^{(b) (6)} was a virologic success at WK 96. Viral load data at WK 96 for subject # 94315 with baseline isolates harboring V108I plus V179E substitutions were not provided.

On-therapy isolates from 5 subjects (# [REDACTED]^{(b) (6)}) from Arm 2 of the Encore1 trial developed NNRTI resistance-associated substitutions (K101E, K103N/R, V106M, V179D, G190R, P225H and F227L) during treatment at WKs 12 to 72. The amino acid substitution K103N present in baseline isolates from subject # [REDACTED]^{(b) (6)} persisted in on-therapy isolates and an additional substitution P225H developed in on-therapy isolates at WK 48.

Although, on-therapy isolates from 5 subjects (# [REDACTED]^{(b) (6)}) from Arm 2 of the Encore1 trial developed treatment-emergent NNRTI resistance-associated substitutions during WKs 12 to 72, HIV-1 levels for 3 subjects (# [REDACTED]^{(b) (6)}) were below 200 copies/mL at WK96 and these subjects were considered virologic successes. However, subject # [REDACTED]^{(b) (6)} had HIV-1 RNA levels of 2,227,803 copies/mL at WK 48 and this subject was a virologic failure at WK 48. Subject # [REDACTED]^{(b) (6)} was a virologic failure at WK 48 and viral load data at WK96 were not available for subject # [REDACTED]^{(b) (6)}. Subject # [REDACTED]^{(b) (6)} with treatment-emergent K101 E substitution was a virologic success at WK 48 and a virologic failure at WK 96.

Baseline isolates from 1 subject (# [REDACTED]^{(b) (6)}) enrolled in Arm 2 of the Encore1 trial harbored NRTI resistance-associated substitution T69N. On-therapy isolates from 3 subjects in Arm 2 of the Encore trial developed NRTI resistance-associated substitutions M41L, K65R, and M184 during treatment at Wks 36 to 48. One subject (# [REDACTED]^{(b) (6)}) developed M41L, K65R, and M184 substitutions at WK 36 and isolates from 2 additional subjects (# [REDACTED]^{(b) (6)}) developed M184V substitution at WK 48.

Although, on-therapy isolates from 6 subjects (# [REDACTED]^{(b) (6)}) developed treatment-emergent NNRTI and NRTI resistance-associated substitutions, HIV-1 levels for 2 subjects ([REDACTED]^{(b) (6)}) were below 200 copies/mL at WKs 48 and 96 and these subjects were considered virologic successes. Subject # [REDACTED]^{(b) (6)} was a virologic failure at WK 48 and HIV-1 RNA levels for subject # [REDACTED]^{(b) (6)} at WK 96 were not available. HIV-1 RNA levels for subjects # [REDACTED]^{(b) (6)} and # [REDACTED]^{(b) (6)} were <200 copies/mL at WK 48 and were 3449 and 85300 copies/mL, respectively, at WK 96. Both subjects (# [REDACTED]^{(b) (6)}) were virologic failures at Wk96. HIV-1 RNA levels for subject # [REDACTED]^{(b) (6)} were 227,983 and 126 copies/mL at WKs 48 and 96, respectively. The subject # [REDACTED]^{(b) (6)} was considered a virologic failure at WK 48, but a virologic success at WK 96.

Four subjects were identified as virologic failures at WK 96 in Arm 2 of the Encore1 study (# [REDACTED]^{(b) (6)}). NNRTI and NRTI resistance-associated

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substitutions were not identified at any time points in isolates from subjects # (b) (6)) in Arm 2 of the Encore1 trial.

Treatment-emergent NNRTI substitutions (V90I, K101E, K103N/R, V106M, V108I, E138A, V179D/E, G190A/R, P225H and F27L) developed in isolates from 4 subjects in Arm 1 and 5 subjects in Arm 2 of the Encore1 trial. Similarly, NRTI resistance-associated substitution, M184V emerged in on-therapy isolates from 2 subjects in Arm 1 and 3 subjects in the Arm 2 of Encore1 trial. In addition, an M184V substitution present in baseline isolates from 1 subject in the Arm 1 of Encore trial persisted at Wks 36 to 72.

Polymorphisms at 132I/M/L, 135I/K/L/R/T, 228L/H/R and 238K/N/R/Q were examined for possible association with virologic failure.

Polymorphisms at amino acid position 132 and 228 were not observed in baseline and on-therapy isolates from virologic failure subjects in Arm 1 and Arm 2 of the Encore1 trial.

135R polymorphisms were present in baseline and on-therapy isolates at Wks 48 and 96 from a virologic failure subject # (b) (6) in Arm 1 of the Encore1 trial. Similarly, 135K/R polymorphisms were also present in on-therapy isolates at WK 48 from another virologic failure subject # (b) (6) in Arm 1 of the Encore1 trial. In addition, 135T polymorphism was present in baseline isolate from other virologic failure subject # (b) (6) in Arm 1 of the Encore1 trial. Additionally, 135T polymorphism was present in baseline and WK 60 isolates from subject # (b) (6) and in baseline isolates from subject # (b) (6) in Arm 1 of the Encore1 trial. Both subjects (# (b) (6)) had HIV-1 RNA levels below 200 copies/mL at WK 96 and are considered virologic successes.

Polymorphisms 135L/T were observed in baseline isolates from 3 subjects (# (b) (6)) in Arm 2 of the Encore trial. The 135T polymorphism persisted in WK 60 isolates from only 1 subject # (b) (6). These 3 subjects with baseline 135L/T polymorphisms had HIV-RNA levels <200 copies/mL at WK 96 and are considered virologic success.

Polymorphism 238R was observed in baseline isolates from virologic failure subjects in Arm 1 of the Encore1 trial.

Polymorphisms 238K/R/N were not observed in baseline and on-therapy isolates from virologic failure subjects in Arm 2 of the Encore1 trial.

Novel amino acid substitutions were not detected in isolates from subjects in Arm 1 and Arm 2 of the Encore1 trial. Substitutions identified by the sponsor in red font in Excel sheets are present in baseline isolates and may not be treatment associated.

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Microbiology Label (02/13/17)

The sponsor's proposed label is a compilation of data from the individual drug labels. The strikethrough is shown in red font and DAVP edit in blue font.

12. CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

antiviral drugs (b) (4) fixed dose combination of (b) (4) with antiviral activity against HIV-1 [see *Microbiology (12.4)*].

12.4 Microbiology

Mechanism of Action

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Efavirenz: (b) (4) is an NNRTI of HIV-1. Efavirenz activity is mediated predominantly by noncompetitive inhibition of HIV-1 reverse transcriptase (RT). HIV-2 RT and human cellular DNA polymerases α , β , γ , and δ are not inhibited by efavirenz.

Lamivudine: (b) (4) is a synthetic nucleoside analogue with activity against HIV-1 and HBV. Intracellularly, (b) (4) is phosphorylated to its active 5'-triphosphate metabolite, lamivudine triphosphate (3TC-TP). The principal mode of action of 3TC-TP is the inhibition of HIV-1 (b) (4) reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue.

Tenofovir Disoproxil Fumarate: (b) (4)
(b) (4) is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. (b) (4) requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase and HBV (b) (4) reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α , β , and mitochondrial DNA polymerase γ .

Antiviral Activity

Efavirenz: The concentration of efavirenz inhibiting replication of wild-type laboratory adapted strains and clinical isolates in cell culture by 90 to 95% ($EC_{90\text{ to }95}$) ranged from 1.7 to 25 nM in lymphoblastoid cell lines, peripheral blood mononuclear cells (PBMCs), and macrophage/monocyte cultures. (b) (4) demonstrated antiviral activity against clade B and most non-clade B isolates (subtypes A, AE, AG, C, D, F, G, J, N), but had reduced antiviral activity against group O viruses. (b) (4)

Lamivudine:- (b) (4)
(b) (4) The antiviral activity of (b) (4) against HIV-1 was assessed in a number of cell lines (including monocytes and fresh human peripheral blood lymphocytes (PBMCs)) using standard susceptibility assays. EC_{50} values were in the range of (b) (4). (1 μ M = 0.23 mcg/mL). The median EC_{50} values of (b) (4) were 60 nM (range: 20 to 70 nM), 35 nM (range: 30 to 40 nM), 30 nM (range: 20 to 90 nM), 20 nM (range: 3 to 40 nM), 30 nM (range: 1 to 60 nM), 30 nM (range: 20 to 70 nM), 30 nM (range: 3 to 70 nM), and 30 nM (range: 20 to 90 nM) against HIV-1 clades A-G and group O viruses (n = 3 except n = 2 for clade B), respectively. The EC_{50} values against HIV-2 isolates (n = 4) ranged from (b) (4) (b) (4) 3 to 120 nM in PBMCs. (b) (4) was not antagonistic to all tested anti-HIV agents.

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Ribavirin (50 microM) used in the treatment of chronic HCV infection decreased the anti-HIV-1 activity of (b) (4) by 3.5 fold in MT-4 cells.

Tenofovir Disoproxil Fumarate: The antiviral activity of tenofovir against laboratory and clinical isolates of HIV-1 was assessed in lymphoblastoid cell lines, primary monocyte/macrophage cells and peripheral blood lymphocytes. The EC₅₀ (50% effective concentration) values for tenofovir were in the range of 0.04 µM to 8.5 µM. (b) (4)

(b) (4) Tenofovir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC₅₀ values ranged from 0.5 µM to 2.2 µM) and strain specific activity against HIV-2 (EC₅₀ values ranged from 1.6 µM to 5.5 µM). Please see the full prescribing information for VIREAD for information regarding the inhibitory activity of lamivudine against HBV.

Resistance

Efavirenz: In cell culture, HIV-1 isolates with reduced susceptibility to (b) (4)z (>380-fold increase in EC₉₀ value) emerged rapidly in the presence of drug. Genotypic characterization of these viruses identified single amino acid substitutions L100I or V179D, double substitutions L100I/V108I, and triple substitutions L100I/V179D/Y181C in (b) (4) reverse transcriptase.

(b) (4)

Clinical isolates with reduced susceptibility in cell culture to efavirenz have been obtained. One or more RT substitutions at amino acid positions A98, L100, K101, K103, V106, V108, Y188, G190, P225, F227 and M230 were observed in patients failing treatment with (b) (4) in combination with indinavir, or with (b) (4) plus zidovudine (b) (4). The K103N substitution was the most frequently observed.

Lamivudine: (b) (4) resistant variants of HIV-1 have been selected in cell culture.

Genotypic analysis showed that resistance was predominantly due to a methionine to valine (b) (4) or (b) (4) isoleucine (M184V/I) substitution in reverse transcriptase. (b) (4)

Tenofovir Disoproxil Fumarate: HIV-1 isolates with reduced susceptibility to tenofovir have been selected in cell culture. These viruses expressed a K65R substitution in reverse transcriptase and showed a 2- to 4-fold reduction in susceptibility to tenofovir. In addition, a K70E substitution in HIV-1 reverse transcriptase has been selected by tenofovir and results in low-level reduced susceptibility to tenofovir. K65R substitutions developed in some subjects failing a tenofovir disoproxil fumarate regimen.

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Cross-Resistance

Efavirenz: Cross-resistance among NNRTIs has been observed. Clinical isolates previously characterized as (b) (4)-resistant were also phenotypically resistant in cell culture to (b) (4) delavirdine and (b) (4) nevirapine compared to baseline. (b) (4) Delavirdine- and/or nevirapine (b) (4)-resistant clinical viral isolates with NNRTI resistance-associated substitutions (A98G, L100I, K101E/P, K103N/S, V106A, Y181X, Y188X, G190X, P225H, F227L, or M230L) showed reduced susceptibility to EFV (b) (4) in cell culture. Greater than 90% of NRTI-resistant clinical isolates tested in cell culture retained susceptibility to (b) (4).

Lamivudine: Cross-resistance among NRTIs has been observed. (b) (4)-resistant HIV-1 isolates were cross-resistant in cell culture to didanosine (ddI). Cross-resistance is also expected with abacavir and emtricitabine as these select M184V substitutions.

Tenofovir Disoproxil Fumarate: Cross-resistance among NRTIs has been observed. The K65R and K70E substitutions selected by tenofovir are also selected in some HIV-1 infected (b) (4) subjects treated with abacavir or didanosine (b) (4). HIV-1 isolates with the K65R substitution also showed reduced susceptibility to (b) (4). HIV-1 isolates from subjects (N=20) whose HIV-1 expressed a mean of 3 zidovudine-associated RT amino acid substitutions (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N) showed a 3.1-fold decrease in the susceptibility to tenofovir. Subjects whose virus expressed an L74V substitution without zidovudine resistance-associated substitutions (N=8) had reduced response to VIREAD. Limited data are available for patients whose virus expressed a Y115F substitution (N=3), Q151M substitution (N=2), or T69 insertion (N=4), all of whom had a reduced response.

RECOMMENDATIONS

With respect to virology, NDA 208255 is approvable.

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Microbiologist

CONCURRENCES:

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Appendices

Appendix 1: Viral Load Data for Subjects enrolled in Arm 1 (EFV 400 mg) of Encore1 Trial with NNRTI and NRTI Resistance-associated Substitutions at Baseline and On-therapy

Subject	Viral Load (HIV-1 copies/mL)		
	WK 0	WK 48	WK 96
(b) (6)	2367714	232	632
	379692	524	220
	70312	40	40
	65347	40	986
	3820	40	40
	129038	84	40
	2141	40	40
	97300	40	40
	41759	40	40
	1669	40	505
	274336	40	40
	87180	40	40
	34361	40	40
	92292	40	40
	84571	40	40
	17415	40	114
	30016	40	40
	18065	40	40
	23506	40	40
	39625	40	40

Appendix 2: Viral Load Data for Subjects Enrolled in Arm 2 (EFV 600 mg) of Encore1 Trial with NNRTI and NRTI Resistance-associated Substitutions at Baseline and On-therapy

Subject	Viral Load		
	WK 0	WK 48	WK 96
(b) (6)	416226	ND	ND
	65810	40	3449
	262753	139	40
	444837	40	50
	4109	62	40
	192166	ND	ND
	167269	40	40
	86202	40	40

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(b) (6)	35471	40	96
	270039	50	40
	24000	87	40
	12799	40	40
	125680	40	40
	72131	30749	85300
	133894	40	40
	304301	227893	126
	1223249	40	40
	47649	40	40
	35174	40	40
	123407	40	40

ND= no data

Appendix 3: List of WK 48 Virologic Failure Subjects in Arm 1 and Arm 2 of Encore1 Trial

Arm 1 (EFV 400 mg)	Viral Load at WK 48	Arm 2 (EFV 600 mg)	Viral Load (copies/mL) at WK 48
(b) (6)	5556	(b) (6)	>200
	232		577
	524		>200
	42283		3295
	967		8225
	3365		209196
	602266		1860
	3452		30749
	8029		227893
			430

Appendix 4: List of WK 96 Virologic Failure Subjects in Arm 1 and Arm 2 of Encore1 Trial

Arm 1 (EFV 400 mg)	Viral Load at WK 96	Arm 2 (EFV 600 mg)	Viral Load at WK 96
(b) (6)	632	(b) (6)	611
	220		3449
	986		3075
	869		85300
	349		

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(b) (6)	505		
	1076		
	61884		
	81301		
	266		

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/s/

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02/14/2017

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