Phase II Study of Vicriviroc versus Efavirenz (both with Zidovudine/Lamivudine) in Treatment-Naive Subjects with HIV-1 Infection

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Background. Vicriviroc (VCV) is a CCR5 antagonist with nanomolar activity against human immunodeficiency virus (HIV) replication in vitro and in vivo. We report the results of a phase II dose-finding study of VCV plus dual nucleoside reverse-transcriptase inhibitors (NRTIs) in the treatment-naive HIV-1–infected subjects.

Methods. This study was a randomized, double-blind, placebo-controlled trial that began with a 14-day comparison of 3 dosages of VCV with placebo in treatment-naive subjects infected with CCR5-using HIV-1. After 14 days of monotherapy, lamivudine/zidovudine was added to the VCV arms; subjects receiving placebo were treated with efavirenz and lamivudine/zidovudine; the planned treatment duration was 48 weeks.

Results. Ninety-two subjects enrolled. After 14 days of once-daily monotherapy, the mean viral loads decreased from baseline values by 0.07 $\log_{10}$ copies/mL in the placebo arm, 0.93 $\log_{10}$ copies/mL in the VCV 25 mg arm, 1.18 $\log_{10}$ copies/mL in the VCV 50 mg arm, and 1.34 $\log_{10}$ copies/mL in the VCV 75 mg arm ($P < .001$ for each VCV arm vs. the placebo arm). The combination-therapy portion of the study was stopped because of increased rates of virologic failure in the VCV 25 mg/day arm (relative hazard [RH], 21.6; 95% confidence interval [CI], 2.8–168.9) and the VCV 50 mg/day arm (RH, 11.7; 95% CI, 1.5–92.9), compared with that in the control arm.

Conclusions. VCV administered with dual NRTIs in treatment-naive subjects with HIV-1 infection had increased rates of virologic failure, compared with efavirenz plus dual NRTIs. No treatment-limiting toxicity was observed. Study of higher doses of VCV as part of combination therapy is warranted.

Increasing rates of resistance, long-term metabolic and end-organ toxicities are driving factors in the develop-

ment of novel agents and regimens for HIV-infected treatment-naive persons [1–9].

C-C motif chemokine receptor 5 (CCR5) is one of 2 clinically important coreceptors used by HIV during CD4 cell entry. CCR5-using (i.e., R5) virus predominates early in infection, with ∼80% of subjects harboring subtype B virus exhibiting CCR5 use exclusively [10–12]. CCR5 has been a target for HIV therapeutics since the observation that congenital absence confers resistance to infection with minimal clinical sequelae [13, 14]. Moreover, CCR5Δ32 heterozygotes have evidence of slowed HIV disease progression [15–17].

Theoretically, the use of CCR5 antagonists may select for CXCR4-using (i.e., X4) viral populations, which have been associated with rapid disease progression [18]. Whether emergence of X4 virus accelerates disease progression or is a manifestation of advancing disease is unclear [19–21]. Vicriviroc (VCV [SCH-D, SCH 417690]) is a small-molecule antagonist of CCR5 that is potent at nanomolar concentrations, is highly specific for the primate CCR5 receptor, and has excellent oral bioavailability with minimal preclinical evidence of cardiac toxicity [22–24].

In a phase 1 study involving HIV-infected treatment-naive subjects, VCV monotherapy was associated with a decrease of $1.6 \log_{10}$ HIV RNA copies/mL at 14 days and was found to be safe at twice-daily doses of 10 mg, 25 mg, and 50 mg [24]. Given the linear and predictable pharmacokinetic profile of VCV and its mean half-life of 16–17 h, once-daily dosing was selected for further evaluation.

SUBJECTS, MATERIALS, AND METHODS

Study design. This was a phase II, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the efficacy of 3 once-daily doses of VCV in treatment-naive subjects infected with R5 virus. Subjects were excluded if they had received antiretroviral therapy (ART) for a cumulative duration of >2 weeks, if they had ever had X4 or dual/mixed virus (i.e., virus capable of using CCR5 and/or CXCR4; hereafter, "DM virus") recovered, or if they had received ART during the 2-month period before randomization.

After screening, eligible subjects were randomized 1:1:1:1 to receive double-blinded, once-daily VCV (25 mg, 50 mg, or 75 mg) monotherapy or placebo for 14 days. During the subsequent 46-week combination-therapy phase, subjects in the VCV arms continued to receive VCV treatment and lamivudine/zidovudine (3TC/ZDV) twice daily was added, whereas subjects in the placebo arm received open-label efavirenz (EFV) 600 mg daily plus 3TC/ZDV twice daily.

Discontinuation of treatment because of virologic failure was mandated if ≥1 of the following criteria were met: the HIV-1 RNA load was confirmed by retesting to have decreased by <1.0 $\log_{10}$ copies/mL between baseline and week 4, the HIV-1 RNA load was confirmed by retesting to be >400 copies/mL on or after week 20, the HIV-1 RNA load was confirmed by retesting to have become detectable (defined as a load of >400 copies/mL) after a period of undetectability, and/or DM or X4 virus was detected. During the combination-therapy phase, subjects who developed treatment-limiting toxicity or intolerance were allowed to make within-class substitutions (stavudine and nevirapine were provided for treatment-limiting toxicity due to ZDV and EFV, respectively; no substitution was provided for intolerance to VCV). Subjects who experienced virologic failure were discontinued from the study and treated according to standard local HIV therapy guidelines, under direction of treating physicians.

Study population. The study population comprised antiretroviral-naive HIV-infected adults (age, ≥18 years) with a plasma HIV-1 RNA level of ≥5000 copies/mL, as determined by the Amplicor HIV-1 Monitor test, version 1.5 (Roche Molecular Systems), and a CD4 cell count of ≥150 cells/mm^3. HIV-1 from all subjects was tested for phenotypic susceptibility to VCV (defined in terms of the maximum percentage inhibition [MPI] and the IC_{50}) and for tropism. Subjects were excluded from the study if DM or X4 virus was detected by the PhenoSense Entry assay (currently marketed as Trofile [Monogram Biosciences] [25]). Other exclusion criteria included seizure disorder, evidence of seizure-predisposing conditions in the central nervous system, hepatitis C virus coinfection with active viremia, hepatitis B virus coinfection with circulating surface antigen, and pregnancy or breast-feeding. Subjects with baseline genotypic evidence of resistance to EFV, 3TC, or ZDV were excluded. Voluntary written informed consent was obtained from all subjects. Institutional review boards at each site approved the study protocol and documents.

Study procedures. Study visits occurred weekly during the first 4 weeks of treatment, at 4-week intervals during weeks 5–24, and at 8-week intervals during weeks 25–48. CD4 cell counts were measured at weeks 2, 4, 12, 20, 24, 32, 40, and 48. Coreceptor tropism and susceptibility to VCV were measured at baseline, day 1, day 14, week 24, week 48, and at the time of virologic failure or early treatment discontinuation. VCV levels were assessed for population pharmacokinetic analysis at the time of virologic failure and for all subjects remaining on study at the time of discontinuation of the VCV 25 mg arm.

Pregnancy testing was performed for women at baseline and day 1 and was repeated if pregnancy was suspected. CCR5Δ32 genotype was assayed at baseline, and CCR5 mRNA expression was measured at baseline and at day 14 for subjects who consented to genetic testing. Adherence to therapy was measured by pill counts and patient report at each visit.

Information on adverse events and clinical characteristics was collected at each visit. Adverse events were graded according to the toxicity grading scale of the AIDS Clinical Trials Group (available at: http://www3.niaid.nih.gov/research/resources/ DAIDSclinicalresearch/PDF/safety/DAIDSAEGradingTable .pdf). An independent data safety monitoring board (DSMB) monitored the progress and results of the study.

Subjects who met criteria for virologic failure, had treatment-limiting adverse events despite allowable drug substitution, or had DM or X4 virus were discontinued from the study. Investigators were provided data on genotypic and phenotypic resistance (determined by means of the PhenoSense assay [Monogram BioSciences]), coreceptor use, and VCV susceptibility in order to optimize subsequent treatment.

Table 1. Baseline demographic and clinical characteristics of study subjects.

| Characteristic | Total (n = 92) | Vicriviroc arm, by dosage | | | Placebo/ EFV control arm (n = 24) |
		25 mg QD (n = 23)	50 mg QD (n = 22)	75 mg QD (n = 23)	
Age, median (IQR), years	37 (33–41)	37 (33–42)	36 (31–41)	37 (33–40)	38.5 (33–42.5)
Female sex	18 (20)	5 (23)	2 (9)	7 (30)	4 (17)
Race					
White	79 (86)	21 (91)	21 (95)	16 (70)	21 (88)
Black	11 (12)	2 (9)	0	6 (26)	3 (13)
Other	2 (2)	0	1 (5)	1 (4)	0
Ethnicity					
Latino	15 (16)	6 (26)	4 (18)	2 (9)	3 (13)
Non-Latino	77 (84)	17 (74)	18 (82)	21 (91)	21 (88)
HIV-1 RNA load[a]					
Median (IQR), $\log_{10}$ copies/mL	4.79 (4.39–5.12)	4.78 (4.45–5.03)	4.92 (4.43–5.12)	4.80 (4.27–5.12)	4.70 (4.46–5.19)
≥100,000 copies/mL	33 (36)	6 (26)	9 (41)	8 (35)	10 (42)
<100,000 copies/mL	59 (64)	17 (74)	13 (59)	15 (65)	14 (58)
CD4 cell count,[a] median (IQR), cells/mm^3	290 (231–347)	286 (246–334)	292 (229–322)	295 (239–371)	286 (214–363)

NOTE. Data are no. (%) of study subjects, unless otherwise indicated. IQR, interquartile range; QD, once daily.

[a] Comparisons between study arms were made by analysis of variance and the Kruskall-Wallis test. No P values were statistically significant.

mRNA analysis. Peripheral blood was collected in specialized tubes (PAXgene), and RNA was prepared according to the manufacturer's instructions. The quantity and quality of isolated RNA was analyzed using RNA nano chips (Agilent Technologies). TaqMan primers and probes were designed with Universal Probe Library Assay Design software (Roche). Quantitative polymerase chain reaction (PCR) was performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). PCR reactions were prepared using the components from the iScript Custom One-Step reverse transcription (RT)–PCR kit with ROX and assembled according to the manufacturer's instructions (Bio-Rad). Final concentrations of primers and probes were 200 nmol/L and 100 nmol/L, respectively. Probes were labeled with 6-carboxyfluorescein. Each 10-μL PCR reaction contained 2 μL (20 ng) of RNA. RT-PCR reactions were performed in triplicate in a 384-well plate. A eukaryotic 18S rRNA endogenous control probe/primer set (ABI) was used as an internal control for RNA quality. PCR data were quantitated using a 12-point standard curve generated by means of 4-fold serial dilutions of a cDNA containing the gene of interest. CCR5 expression was normalized to 18S RNA levels.

Statistical analysis. All analyses were based on the modified intent-to-treat (ITT) principle (i.e., data for all randomized subjects who received at least 1 dose of a study drug were included). The primary study end point was the mean change in the $\log_{10}$ HIV-1 RNA load from baseline to day 14.

Changes from baseline HIV-1 RNA loads and CD4 cell counts were analyzed using an analysis of variance (ANOVA) model with treatment as the covariate. For subjects with a missing HIV-1 RNA or CD4 cell measurement, the last observation made after baseline was carried forward. The Fisher exact test was used to compare proportions of subjects for whom the HIV-1 RNA load changed by at least 1.0 $\log_{10}$ copies/mL, those who maintained an HIV-1 RNA load of <50 copies/mL, and those who maintained an HIV-1 RNA load of <400 copies/mL. Kaplan-Meier analysis was used to investigate the time to virologic failure, and treatment arms were compared using the logrank test. Cox proportional hazards modeling was used to compare event rates. Association between the reduction in the HIV-1 RNA load by day 14 and long-term response or virologic failure among subjects receiving VCV was also explored using logistic regression. Analyses were conducted using SAS, version 8 (SAS Institute). Analysis of CCR5 expression was performed using Graphpad Prism, and mean values were compared using the Student t test and ANOVA. The study was planned to ensure 90% power to detect a difference of 1.0 $\log_{10}$ copies/mL from the baseline HIV-1 RNA load between the VCV 75 mg arm and the placebo arm, assuming a standard deviation of 0.7 $\log_{10}$ copies/ mL.

RESULTS

Ninety-two subjects entered the study between July 2004 and May 2005. Eighty percent were male, and 86% were white. The median baseline CD4 cell count was 290 cells/mm^3, and the median HIV-1 RNA load was 4.79 $\log_{10}$ copies/mL; 36% of subjects had a baseline HIV-1 RNA load of ≥100,000 copies/mL. Baseline characteristics were balanced across treatment groups (table 1). Sixty-three subjects had samples suitable for CCR5Δ32 mutation assessment. Eight subjects were heterozygous for CCR5Δ32, and the remaining 55 were homozygous for the wild-type allele.

Subject disposition at conclusion of monotherapy. One subject receiving VCV monotherapy was lost to follow-up. Ninety-one subjects completed the monotherapy phase. Eighty-eight subjects (97%) entered the combination-therapy phase. Three subjects receiving placebo elected not to continue into the combination phase.

HIV-1 RNA response at day 14. The mean decrease in the HIV-1 RNA load from baseline to day 14 was 0.93 $\log_{10}$ copies/mL for the VCV 25 mg arm, 1.18 $\log_{10}$ copies/mL for the VCV 50 mg arm, 1.34 $\log_{10}$ copies/mL for the VCV 75 mg arm, and 0.07 $\log_{10}$ copies/mL for the placebo arm ($P < .001$ for each VCV arm vs. the placebo arm, by modified ITT analysis) (figure 1*A*).

Interim analyses: combination-therapy phase. A planned DSMB review at the time of primary end point completion by all subjects noted a trend toward increased rates of persistent viremia of ⩾50 copies/mL in the VCV arms and recommended that all study arms continue to receive therapy. All subsequent decisions were made on the basis of the more rigorous 50 copies/mL threshold for virologic failure. Follow-up review 4 weeks later demonstrated that the rate of virologic failure in the VCV 25 mg arm was greater than that in the control arm (relative hazard [RH], 21.6; 95% confidence interval [CI], 2.8–168.9). The DSMB recommended that the VCV 25 mg arm be terminated. Treatment for all subjects in this arm was unblinded and discontinued regardless of viral load suppression status, and CD4 cell and HIV-1 RNA findings were censored. The median treatment duration at the time of arm discontinuation was 24.4 weeks (range, 7.9–53.4 weeks). The DSMB recommended that the VCV 50 mg and 75 mg arms continue with ongoing monthly monitoring. In subsequent follow-up analysis, the board noted that the rates of virologic failure in the VCV 50 mg arm (RH, 11.7; 95% CI, 1.5–92.9) and the pooled 50 mg and 75 mg arms (RH, 7.8; 95% CI, 1.0–59.9) were greater than that in the control arm and recommended study termination. The sponsor concurred and terminated the study. At the time of termination, the rate of virologic failure in the VCV 75 mg arm was not statistically different from that in the control arm (table 2). The median durations of treatment at the time of discontinuation were 32.7 weeks (range, 1.1–57.4 weeks) in the VCV 50 mg arm, 36.3 weeks (range, 6.3–59.0 weeks) in the VCV 75 mg arm, and 35.8 weeks (duration, 2.0–50.0 weeks) in the control arm. All subjects discontinued study medication at the time of study closure. Data on CD4 cell counts and viral loads were censored upon discontinuation, and subsequent treatment was pursued at the investigator's discretion. Virologic decay curves for all subjects are depicted in figure 1*B*. At study termination, the times to failure were shorter in the VCV 25 mg arm ($P < .001$) and VCV 50 mg arm ($P = .003$) but not the VCV 75 mg arm ($P = .140$), compared with the control arm (figure 2).

Of the 88 treated subjects in the combination-therapy phase, 14 (17%) completed 48 weeks of treatment. Treatment was not completed because of study termination (for 54 subjects [61%]), virologic failure (for 18 [20%]), an adverse event (for 1 [1%]), and withdrawal of consent (for 1 [1%]).

Immunologic and virologic response. At week 24, all treatment groups achieved sizable increases in mean CD4 cell counts; these changes were not significantly different between treatment groups (table 2). The mean reduction in the HIV-1 RNA load between baseline and week 24 was smaller in the VCV 25 mg arm (2.43 $\log_{10}$ copies/mL; $P = .003$) and 75 mg arm (2.65 $\log_{10}$ copies/mL; $P = .02$), compared with the EFV control arm (3.20 $\log_{10}$ copies/mL).

Adherence. Adherence rates were calculated as the percentage of days during the treatment period that were covered by drug exposure, using pill counts and patient self-reports. All subjects reported 100% adherence during the monotherapy phase. During the combination-therapy phase, adherence rates were >98.6%.

Changes in coreceptor use. X4 virus emerged in 8 subjects (DM virus was detected in 7, and X4 virus was detected in 1). Three of the 8 observed changes in tropism occurred in the placebo group without exposure to VCV (all involved DM virus), 1 was observed in the VCV 25 mg group (DM virus), and 4 were observed in the VCV 75 mg group (3 involved DM virus, and 1 involved X4 virus). Most (6 of 8) tropism changes were detected on or before day 14, including the emergence of X4 virus.

Two of 3 subjects with non-R5 virus by day 14 who were receiving VCV, including the subject with X4 virus, experienced no suppression of viral load during the monotherapy period. For 2 subjects in whom changes in coreceptor use were not observed until week 24, the HIV-1 RNA load decreased by >1 $\log_{10}$ copies/mL and the CD4 cell count increased by >150 CD4 cells/mm³ at the time of DM detection (table 3).

Pharmacokinetics. The pharmacokinetic variables C_{min} (trough plasma concentration), C_{max} (peak plasma concentration), and AUC (area under the curve) increased linearly with the VCV dose and were consistent with predictions from phase 1 studies (table 4). C_{min} appeared to correlate with a reduction in the $\log_{10}$ viral load at day 14 (figure 3). Analyses that adjusted for VCV dose revealed that subjects who experienced virologic failure during receipt of combination therapy had lower mean C_{min} values (43.2 vs. 66.2 mg/mL; $P = .08$ for log-transformed comparison) and lower mean AUC values (1896.9 vs. 2788.3 ng·h/mL; $P = .14$ for log-transformed comparison) on day 14, compared with subjects who did not experience virologic failure.

mRNA expression. mRNA samples from whole blood obtained before the study and on day 14 were available for 13 subjects. No difference was observed in mean CCR5 mRNA levels, normalized for 18S RNA, between day 14 and pretreatment samples taken at baseline. This was consistent among subjects treated with VCV (3.70 vs. 3.26; $P = .59$), among subjects who received placebo (3.60 vs. 3.17; $P = .07$), and between treatment groups at either time point ($P = .97$).
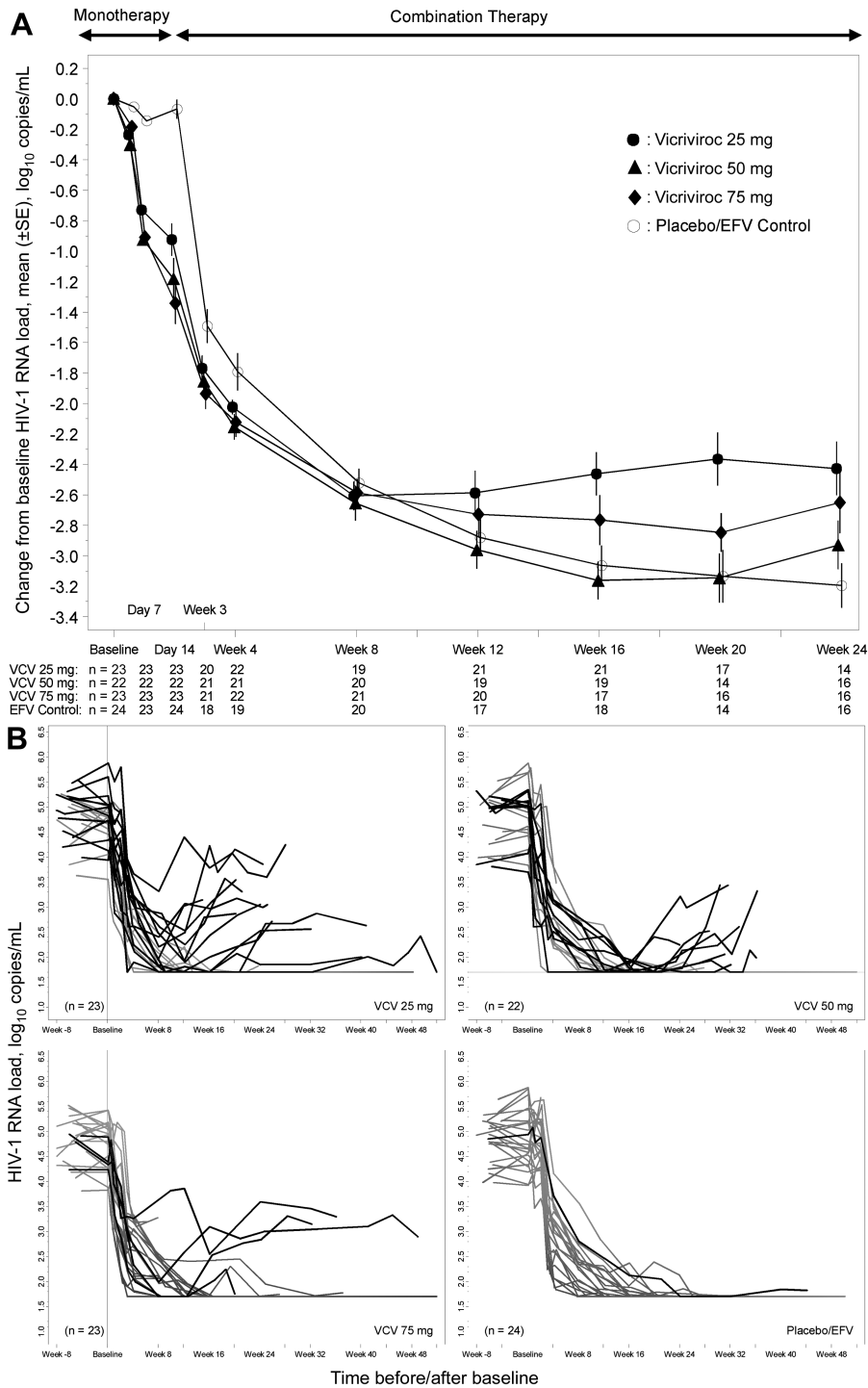
Figure 1. *A,* Change in $\log_{10}$ HIV-1 RNA load in the modified intent-to-treat population of subjects who received vicriviroc (VCV) or placebo/efavirenz (EFV). Space does not permit identification of the plotted data points on day 4 of treatment, which represent sample sizes of 16, 21, 19, and 17 subjects, respectively for the VCV 25 mg, VCV 50 mg, VCV 75 mg, and placebo arms. *B,* Viral load decay plots for individual subjects, by treatment arm. Subjects with virologic failure (defined as achievement of an HIV-1 RNA load of $\geqslant$50 copies/mL on or after week 20) are shown in *black* (on-treatment observed data only).

Resistance. Of the 26 subjects in the VCV arms who experienced virologic failure, 22 had evaluable HIV-1 genotypes at the time of virologic failure. All of the obtainable genotypes demonstrated the M184V, M184I, or M184V/I mutation, consistent with treatment-emergent 3TC resistance. HIV-1 from 1 subject additionally had the M41L mutation. No other treatment-emergent genotypic mutations were observed in the reverse transcriptase gene.

Table 2. Immunologic and virologic outcomes of subjects in the modified intent-to-treat population.

Outcome	Vicriviroc arm, by dosage[a]						Placebo/EFV control arm
	25 mg QD	P	50 mg QD	P	75 mg QD	P	
	n = 23		n = 22		n = 23		n = 24
Increase in CD4 cell count from baseline to day 14, mean ± SD, cells/mm³	24 ± 88	NS	85 ± 57	<.001	90 ± 70	<.001	3 ± 66
Decrease in HIV-1 RNA load from baseline to day 14, mean ± SD, $\log_{10}$ copies/mL	0.93 ± 0.43	<.001	1.18 ± 0.63	<.001	1.34 ± 0.64	<.001	0.07 ± 0.30
Virologic failure[b] as defined by HIV-1 RNA load							
≥400 copies/mL	9 (39)	<.001	2 (9)	NS	3 (13)	NS	0
≥50 copies/mL	13 (56)	<.001	9 (41)	.003	4 (17)	NS	1 (4)
Achieved <50 copies/mL	5 (38)		7 (78)		2 (50)		1 (100)
Never achieved <50 copies/mL	8 (62)		2 (22)		2 (50)		0
Hazard rate for virologic failure, events/person-year (95% CI)	1.35 (0.79–2.30)		0.73 (0.38–1.38)		0.30 (0.12–0.77)		0.07 (0.01–0.39)
Relative hazard for virologic failure (95% CI)[c]	21.6 (2.8–168.9)		11.7 (1.5–92.9)		4.6 (0.5–41.4)		. . .
	n = 14		n = 16		n = 16		n = 16
Increase in CD4 cell count from baseline to week 24, mean ± SD, cells/mm³	73 ± 141	NS	110 ± 103	NS	158 ± 171	NS	102 ± 102
Decrease in HIV-1 RNA load from baseline to week 24, mean ± SD, $\log_{10}$ copies/mL	2.43 ± 0.65	.003	2.93 ± 0.63	NS	2.65 ± 0.80	.02	3.20 ± 0.57

NOTE. Data are number (%) of subjects, unless otherwise indicated. CI, confidence interval; EFV, efavirenz; NS, not significant; QD, once daily.

[a] All P values are for comparisons with the placebo/EFV control arm.

[b] Defined as 1 or more of the following criteria: failure to achieve a reduction of >1 $\log_{10}$ copies/mL in the HIV-1 RNA load by week 4, achievement of an HIV-1 RNA load of ≥400 or ≥50 copies/mL after week 20, and/or achievement of an HIV-1 RNA load of ≥400 or ≥50 copies/mL (depending on the definition used in criterion 2) after the load decreased to <400 or <50 copies/mL, respectively.

[c] Determined by means of a Cox proportional hazards model.

Subjects who went on to experience virologic failure had a mean VCV IC_{50} of 6.97 nmol/L and a mean MPI of 96.02%; values were not significantly different from those for subjects who did not have virologic failure (6.61 nmol/L [P = .78] and 95.95% [P = .91], respectively). Between baseline and the time of virologic failure, the mean MPI decreased (96.02% vs. 90.88%; P = .015) but the IC_{50} was not significantly different (6.97 vs. 7.11 nmol/L; P = .91). The change in the mean MPI from baseline to day 14 was −0.4% (95% CI, −1.6% to 0.8%) among all VCV-treated subjects and −1.18% (95% CI, −2.91% to 0.55%) among subjects who went on to experience virologic failure.

Adverse events. Stavudine was substituted for ZDV in 2 (2%) of 91 subjects because of toxicity; 1 additional subject had a history of ZDV-related nausea and began combination-phase treatment with stavudine/3TC. Nevirapine was substituted for EFV in 3 (13%) of 24 subjects in the control arm because of central nervous system or cutaneous toxicity. During the treat-

ment period, grade 3 adverse events were observed in 2 subjects treated with VCV (1 subject with sinusitis and pyrexia in the VCV 25 mg arm and 1 subject with exacerbation of anal condyloma and depression with suicidal ideation in the VCV 75 mg arm) and in 1 subject in the control arm (major depression). Grade 4 anemia requiring hospitalization and transfusion occurred in 1 subject taking VCV 50 mg plus 3TC/ZDV. One subject discontinued treatment because of severe nausea and vomiting; the subject was taking VCV 75 mg plus 3TC/ZDV.

No seizures were observed during the study. One exacerbation of preexisting Kaposi sarcoma was observed in the control arm, concomitant with immune reconstitution. No grade 3 or 4 increases in liver transaminase levels were observed. A single subject with a normal bilirubin level at study entry developed a transient grade 3 elevated bilirubin level without clinical or other laboratory sequellae. The subject continued to receive study medication, and the bilirubin level decreased to a grade of 2. Adverse events observed at a rate of ≥10% among subjects re-
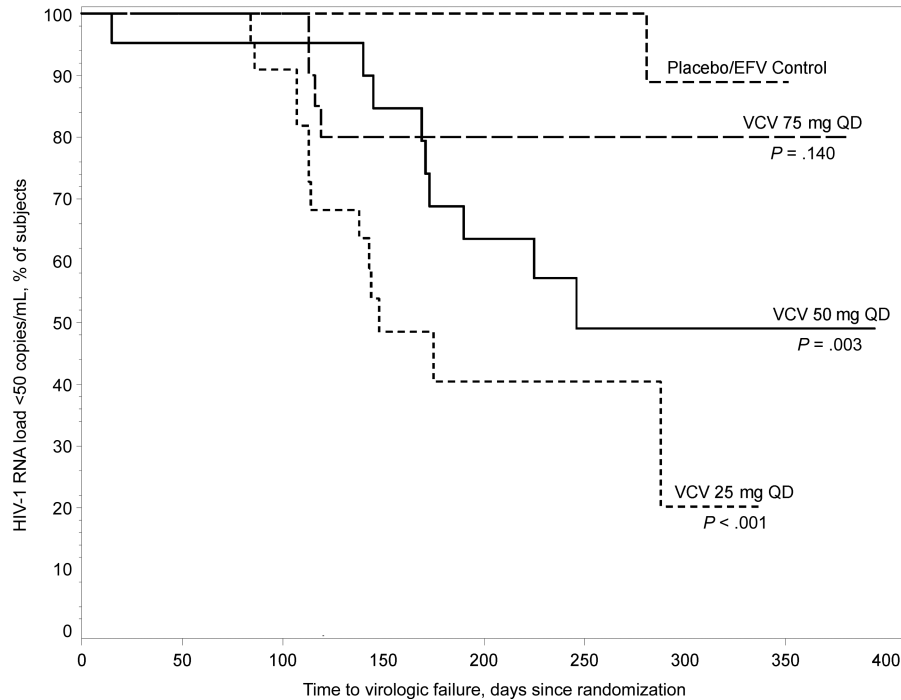
Figure 2. Kaplan-Meier estimates of times-to-virologic failure, defined as achievement of an HIV-1 RNA load of ⩾50 copies/mL on or after week 20, among subjects who received vicriviroc (VCV) or placebo/efavirenz (EFV). Absolute hazard rates for virologic failure and relative hazards are listed in table 2. *P* values are for pairwise comparisons of each VCV arm with the placebo/EFV arm.

ceiving VCV at any dose included nausea, fatigue, headache, diarrhea, nasopharyngitis, upper abdominal pain, anorexia, dizziness, and vomiting. None of these rates of adverse events were significantly different from those in the control group.

DISCUSSION

Despite initial robust decreases in the HIV-1 RNA loads and increases in CD4 cell counts in all VCV arms during the 2-week monotherapy lead-in period, persistent or recurrent detectable viremia was found in the combination-therapy arms containing lower-dose VCV and prompted early termination of the study. Subjects with virologic failure appeared to segregate into 2 subsets: those experiencing virologic failure coincident with a shift in coreceptor tropism use and those with virologic failure in the absence of a tropism shift. Antiretroviral adherence, as measured by pill count and patient self-report, did not account for the virologic failures.

Table 3. Clinical course of 8 subjects in whom HIV-1 using CXCR4 coreceptor only (X4) and HIV-1 capable of using either CCR5 or X4 coreceptor (DM) emerged during the study.

| | | Baseline value | | Tropism change | | Change from baseline value to value at detection of tropism change | |
| | | | | | | | |
Subject	Study arm	HIV-1 RNA load, $\log_{10}$ copies/mL	CD4 cell count, cells/mm^3	Time to detection	New tropism	HIV-1 RNA load, $\log_{10}$ copies/mL	CD4 cell count, cells/mm^3
1	Placebo	5.73	270	Day 14	DM	−0.28	−59
2	Placebo	4.64	302	Day 14	DM	+0.13	+38
3	Placebo	4.57	138	Day 14	DM	+0.10	+29
4	VCV 25 mg	4.72	286	Day 14	DM	−0.67	−11
5	VCV 75 mg	5.04	308	Day 4	DM	−0.09	0
6	VCV 75 mg	4.95	394	Day 14	X4	+0.04	+72
7	VCV 75 mg	4.23	248	Week 24	DM	−1.39	+248
8	VCV 75 mg	4.88	188	Week 24	DM	−1.89	+161

NOTE.   CCR5-using HIV-1 was detected in all subjects at baseline and at the time of screening. VCV, vicriviroc.

**Table 4. Pharmacokinetic characteristics of vicriviroc (VCV), by dose.**

Characteristic	VCV 25 mg (n = 21)	VCV 50 mg (n = 19)	VCV 75 mg (n = 21)
C_{min}, median (range), ng/mL	23.8 (11.2–60.2)	44.1 (17.4–102.2)	77.9 (27.2–249.8)
C_{max}, median (range), ng/mL	123.6 (65.4–149.4)	245.9 (175.9–373.2)	370 (207.7–495.3)
AUC, median (range), ng·h/mL	1095 (682.3–1853)	2104 (1560–3615)	3545 (2097–8276)

NOTE. AUC, area under the curve; C_{max} peak plasma concentration; C_{min}, trough plasma concentration.

CCR5 receptor antagonists do not appear have significant antiviral activity against DM or X4 virus [26]. In this study, emergence of detectable DM or X4 virus was associated with minimal viral decay during monotherapy and with emergent resistance (due to the M184V mutation) during combination therapy, which is typical of subjects treated with dual nucleoside reverse-transcriptase inhibitors (NRTIs; 2 of 2 subjects in this study). Clonal analysis of maraviroc-treated subjects has suggested that early detection of X4 virus (i.e., by day 14 of treatment) most likely represents preexisting X4 populations whose sizes at baseline were less than the threshold of detection for the then-available assay [27]. More-sensitive assays to assess baseline coreceptor use may help identify a more R5-homogeneous treatment population; however, it is increasingly clear that coreceptor tropism evolves fluidly both in the presence and absence of CCR5 antagonist use. In the current study, 3 subjects with R5 virus at screening and baseline had detectable DM virus by day 14 despite only receiving placebo.

In the absence of a change in coreceptor use, a number of other mechanisms of viral escape can be postulated. IC_{50} values do not appear to adequately describe the observed apparent resistance to VCV, consistent with in vitro evidence of on-treatment selection of strains able to bind and accomplish fusion and entry via the CCR5-receptor/CCR5-antagonist complex [28, 29]. Baseline MPIs, more consistent with these mechanistic observations, also were not associated with virologic failure. Decreased MPIs were observed at virologic failure, further validating MPI as the more-relevant resistance measure. Envelope sequencing work by others suggests that resistance may be mediated by critical changes in V3-loop and extra V3-loop sequences, distinct from those associated with coreceptor tropism [30]. Further envelope sequence analyses of subjects with virologic failure are ongoing.

It has been suggested that the 14-day monotherapy period generated VCV resistance, leading to increased failure during combination therapy. This theory is refuted by the
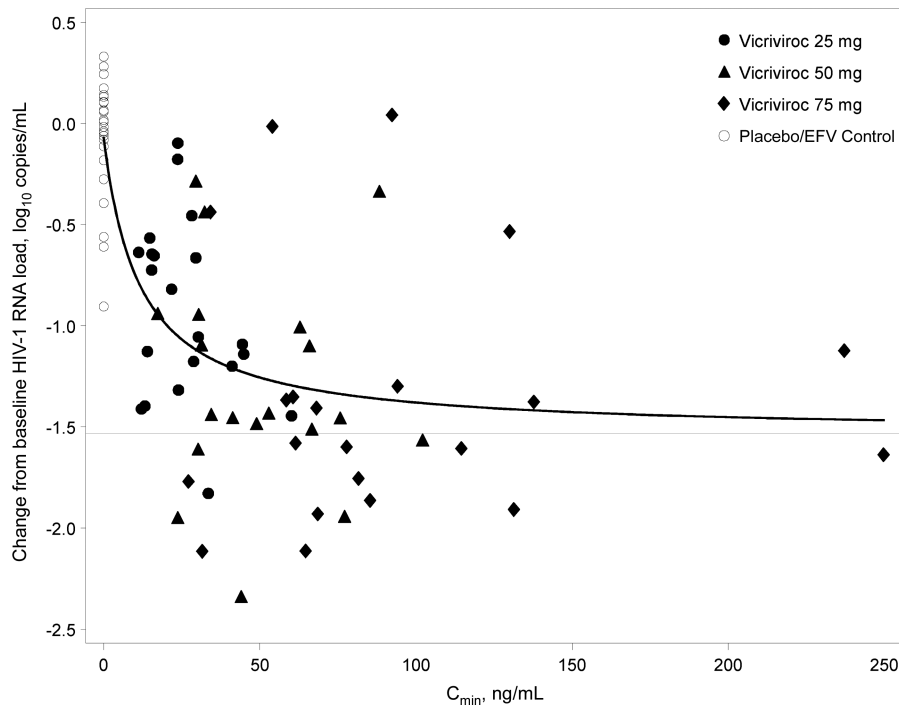
Figure 3. Change in HIV-1 RNA load from baseline to day 14, by trough concentration (C_{min}) of vicriviroc, among subjects who received vicriviroc or placebo/efavirenz (EFV). Analysis was performed using an Emax model with SAS, version 9.1 (SAS Institute), and PROC NLIN. Emax = -1.4649 ± 0.1960 $\log_{10}$ copies/mL. The C_{min} required to achieve 50% of Emax was 11.624 ± 6.32 ng/mL.

nonsignificant mean difference of MPIs between baseline and day 14.

In vitro evidence suggests that intersubject variability in CCR5 cell-surface expression may have important implications for susceptibility to CCR5 inhibitors [31]. CCR5 mRNA was not significantly changed during the monotherapy phase. The power of this observation is limited, however, as we did not directly measure cell-surface CCR5 levels. Preformed vacuolized CCR5 may be externalized to the cell surface in the presence of a CCR5 antagonist and may confound the anticipated correlation between CCR5 mRNA and cell-surface expression. We also did not assess CCR5 expression during combination therapy.

CCR5/CXCR4 independence, rarely observed in pathogenic clinical isolates, has been observed in vitro [32–35] and is not likely to be a clinically important mode of CCR5 inhibitor escape. Although the precise mechanism of virologic failure remains unclear, the high rate of emergence of the M184V mutation in all virologic failures is indicative of inadequate inhibition of viral replication as contributed to by VCV as a third agent at the doses and frequency used in the present study.

Population pharmacokinetic studies performed during post hoc analysis demonstrated that plasma VCV concentrations were consistent with those observed in phase 1 studies [24]. Calculated VCV C_{min} values appeared to be associated with the magnitude of 14-day HIV-1 RNA load decline, although maximization of the dose-response effect was not clearly seen (figure 3).

VCV was well tolerated, with 1 discontinuation because of an adverse event (altered mental status, nausea and vomiting) and 1 grade 4 toxicity (anemia), both thought to be unrelated to VCV. No significant differences in adverse events, laboratory findings, electrocardiogram findings, or other safety parameters were observed between study arms. Seizures, QTc prolongation, and grade 3/4 elevations in transaminase levels were not observed. A VCV study involving treatment-experienced subjects raised concerns about oncogenic properties of the CCR5-antagonist class [36, 37]. No treatment-emergent malignancies were reported in VCV-treated subjects in this study. However, the duration of subject follow-up was shorter, and the median baseline CD4 cell counts were higher than those in the treatment-experienced trial (290 vs. 146 cells/mm^3), limiting the robustness of that observation [38].

Lack of a plateau in the dose response and a wide baseline IC_{50} variability suggest that higher doses of VCV may be required to maximize virologic suppression. Reluctance to study higher doses of VCV, owing to animal seizure activity at levels >10-fold higher than the plasma levels expected and observed in the current study, has been tempered by the absence of neurotoxicity in the clinical database. Additional work has increased confidence in the safety of higher doses, currently in advanced-phase trials [39].

In conclusion, this study demonstrated that, at the doses studied, VCV possesses potent antiviral activity and is associated with a dose-related increase in CD4 cell count. VCV was safe and well tolerated. At once-daily doses of 25 mg and 50 mg, VCV plus dual NRTI therapy was not as effective as EFV plus dual NRTI therapy for viral suppression. Optimization of the role of VCV in combination antiretroviral therapy and characterization of resistance mechanisms require further study.

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