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Antiviral Activity, Pharmacokinetics, and Safety of BMS-488043, a Novel Oral Small-Molecule HIV-1 Attachment Inhibitor, in HIV-1-Infected Subjects▽

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BMS-488043 is a novel and unique oral small-molecule inhibitor of the attachment of human immunodeficiency virus type 1 (HIV-1) to CD4$^+$ lymphocytes. The antiviral activity, pharmacokinetics, viral susceptibility, and safety of BMS-488043 were evaluated in an 8-day monotherapy trial. Thirty HIV-1-infected study subjects were randomly assigned to sequential, safety-guided dose panels of 800 and 1,800 mg BMS-488043 or a matched placebo in a 4:1 ratio, and the drug was administered every 12 h with a high-fat meal for 7 days and on the morning of day 8. Dose-related, albeit less-than-dose-proportional, increases in plasma BMS-488043 concentrations were observed. Mean plasma HIV-1 RNA decreases from the baseline for the BMS-488043 800- and 1,800-mg dose groups on day 8 were 0.72 and 0.96 $\log_{10}$ copies/ml, respectively, compared with 0.02 $\log_{10}$ copies/ml for the placebo group. A lower baseline BMS-488043 50% effective concentration (EC_{50}) in the active-treatment groups was predictive of a greater antiviral response. Although absolute drug exposure was not associated with an antiviral response, the trough concentration (C_{trough}), adjusted by the baseline EC_{50} (C_{trough}/EC_{50}), was associated with antiviral activity. During dosing, four subjects experienced >10-fold reductions in viral susceptibility to BMS-488043, providing further support of the direct antiviral mechanism of BMS-488043. BMS-488043 was generally safe and well tolerated. These results suggest that further development of this novel class of oral HIV-1 attachment inhibitors is warranted.

There is a continuing need for new classes of antiretroviral drugs, driven by increasing concerns over human immunodeficiency virus type 1 (HIV-1) resistance to existing medications, the requirement for lifelong antiretroviral therapy for HIV-1-infected individuals, and the goal of minimizing toxicity (2, 3). The process of HIV-1 entry into host cells offers considerable potential for therapeutic intervention, with viral entry proceeding through multiple sequential steps involving attachment, coreceptor binding, and fusion (8, 13). The early step of viral entry into the host cell is accomplished through binding of the viral envelope glycoprotein complex gp160 to the cellular receptor, CD4. This attachment is followed by conformational changes of the gp160 external glycoprotein portion, gp120, which facilitates the second step involving binding to a cellular coreceptor, usually the chemokine receptor CCR5 or CXCR4. Coreceptor binding, in turn, facilitates a large conformational change and initiates the final entry event involving the gp160 transmembrane glycoprotein gp41, which mediates the fusion of viral and host cell membranes (8, 13).

There are two compounds representing two classes of entry inhibitors currently approved for clinical use, maraviroc, a CCR5 antagonist that targets coreceptor binding, and enfuvirtide, an injectable peptide that inhibits the final step of membrane fusion (1, 8). While they are effective, the use of these existing classes has been limited. The need for laborious and expensive tropism testing and the concern over selecting CXCR4-tropic virus have impacted maraviroc use (2), while the inconvenient route of administration, related injection site side effects, and the availability of more convenient options have severely limited the use of enfuvirtide (12). HIV-1 attachment inhibitors are a new class of selective small-molecule inhibitors of HIV-1 that bind specifically to gp120 and block attachment to CD4$^+$ lymphocytes (5, 10). Unlike the activity of CCR5 antagonists, that of attachment inhibitors is independent of human chemokine coreceptor binding and persists irrespective of viral tropism or the host cell phenotype (5, 7, 10). Furthermore, since attachment inhibitors target a viral protein rather than a host chemokine receptor, they are not expected to impact human immune responses.

The development of BMS-378806, a prototype attachment inhibitor, established gp120 as a viable target for small-molecule inhibitors (5). This compound exhibited some favorable pharmacokinetic traits, such as low protein binding, good oral

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bioavailability in animal models, and a good safety profile, in preclinical testing (10). However, the compound had less-than-optimal pharmacokinetic characteristics overall, such as a short half-life ($t_{1/2}$) (16), which suggested that the drug would not achieve target exposures in humans at acceptable doses and intervals. BMS-488043 is a related compound with improved *in vitro* antiviral activity and a longer $t_{1/2}$ than BMS-378806 in preclinical studies (11, 14, 17). BMS-488043 exhibits potent antiviral activity against macrophage-, T-cell-, and dual-tropic HIV-1 laboratory strains (B subtype) and potent antiviral activity against a majority of subtype B and C clinical isolates, with median 50% effective concentrations (EC_{50}) of 36.5 and 61.5 nmol/liter, respectively (11). Data from a limited number of clinical isolates showed that BMS-488043 exhibited a wide range of activity against the A, D, F, and G subtypes, with no activity observed against three subtype AE isolates (11). The safety, tolerability, and pharmacokinetics of BMS-488043 were previously evaluated in two placebo-controlled studies with healthy non-HIV-infected adults (6). BMS-488043 was generally safe and well tolerated, with no serious adverse events (AEs) in single- and multiple-dose studies. Systemic exposure from repeat dosing was generally dose proportional (400 to 1,200 mg with a high-fat meal and 400 to 800 mg with a light meal), with a limited increase in exposure at higher doses. Based on the findings in healthy adults, a dose of 800 mg twice daily with a high-fat meal was expected to provide minimum concentrations adequate to suppress HIV-1 isolates.

In this 8-day monotherapy trial, the antiviral activity, pharmacokinetics, and safety of BMS-488043 were evaluated in HIV-1-infected subjects. We also evaluated viral susceptibility to BMS-488043 and blood levels of BMS-488043 as predictors of antiviral activity and assessed the development of resistance to BMS-488043 during dosing.

(Part of this research was presented as an abstract at the 11th Conference on Retroviruses and Opportunistic Infections, 8 to 11 February 2004, San Francisco, CA.)

MATERIALS AND METHODS

Study design and patients. This was a randomized, double-blind, placebo-controlled, sequential, ascending multiple-dose study conducted with HIV-1-infected subjects. We randomly assigned 15 subjects to each of two sequential dose panels of 800 and 1,800 mg BMS-488043 (Bristol-Myers Squibb, Princeton, NJ) provided as 200-mg BMS-488043 capsules or a matched placebo in a 4:1 ratio, with treatment administered orally every 12 h with a high-fat meal (945 kcal, 55 g fat) on days 1 to 7 and on the morning of day 8. Subjects were not enrolled for the 1,800-mg dose until safety data evaluated through day 8 for at least 10 subjects confirmed the safety and tolerability of the 800-mg dose. There was no intrasubject dose escalation. The study was conducted in accordance with Good Clinical Practice and the ethical principles of the Declaration of Helsinki. The protocol was approved by the institutional review board at each study site. All subjects provided written informed consent prior to participation in the study.

Eligible subjects were HIV-1-infected male or female adults at least 18 years of age with a $CD4^+$ T-cell count of at least 250 cells/μl and a plasma HIV-1 RNA level of 5,000 to 500,000 copies/ml (Roche Amplicor Assay, version 1.5) within 12 weeks prior to and at the time of a screening visit. Subjects did not receive antiretroviral therapy for at least 16 weeks prior to study participation and were otherwise medically stable as determined by physical, 12-lead electrocardiogram (ECG), and laboratory examinations. Women with childbearing potential were required to have a negative serum or urine pregnancy test within 24 h prior to taking the study medication. Prohibited therapies included the use of any agent known to affect hepatic metabolism or compete with BMS-488043 for CYP3A4 metabolism, the use of any agent known to affect gastrointestinal motility within 1 week of enrollment, and the use of any other drug or herbal preparation within 1 week of enrollment unless approved by the investigator and the study medical monitor.

Evaluations. Physical examination, vital signs, 12-lead ECG, clinical laboratory testing, urine drug screening, pregnancy testing for women, and blood sampling for HIV-1 RNA analysis and $CD4^+$ and $CD8^+$ T-cell counts were conducted at screening (within 30 days prior to study enrollment) and at selected time points throughout the study. Blood samples were taken before entry on days −3 to −1 and prior to dosing on days 1 to 8 for plasma HIV-1 RNA analysis. In addition, blood samples taken on days 1 and 8 were used for $CD4^+/CD8^+$ analyses and to examine viral susceptibility to BMS-488043. Viral drug susceptibility was measured as the EC_{50} of the drug, as described below. Plasma samples for pharmacokinetic assessments were taken before dose administration on days 1 to 8 and also at 1, 2, 3, 4, 6, 8, and 12 h after dose administration on days 1 and 8. During a posttreatment follow-up from day 9 to day 14, subjects had additional blood samples taken for HIV-1 RNA, pharmacokinetic, and $CD4^+/CD8^+$ analyses. AE monitoring was conducted from the first dose administration to study discharge, and monitoring for serious AEs (SAEs) was continued until 30 days after study discharge.

Pharmacokinetic analyses. Blood samples were collected by indwelling catheter or direct venipuncture into tripotassium EDTA-coated tubes from which plasma samples were prepared within 60 min of collection and stored at or below −20°C until ready for analysis. Approximately 350 ml of blood was drawn from each subject during the study. Plasma samples were analyzed at a single central site (Charles River Laboratories, Worcester, MA) using a validated liquid chromatography-mass spectrometry method for BMS-488043 concentrations (4). All samples from any given subject were analyzed in a single run. The standard curves were well fitted by a $1/x$-weighted quadratic equation over a concentration range of 1.00 to 1,000 ng/ml and were used to define the quantifiable limits for study samples. Estimates of within-run and between-run assay variabilities for quality control samples were calculated using one-way analysis of variance, and the coefficient of variation (CV) was no greater than 13.9%, with deviation from the nominal concentrations of no more than ±7.4%. The plasma concentration-time data for BMS-488043 were analyzed by noncompartmental methods (Kinetica, version 4.2; Thermo Electron Corporation, Philadelphia, PA) and were based on experimental observations of the peak plasma concentration (C_{max}), the time to C_{max} (T_{max}), and the trough plasma drug concentration measured predose in the morning (C_{trough}). The area under the plasma concentration-time curve over one dosing interval (12 h) [$AUC_{(TAU)}$] was calculated using the mixed log-linear trapezoidal method. The geometric mean of the C_{trough} values collected on days 6 to 8 was calculated and used as the estimate of C_{trough} at steady state ($C_{trough\ D6\text{-}8}$). The accumulation index (AI) was defined as the ratio of $AUC_{(TAU)}$ at steady state to $AUC_{(TAU)}$ after the first dose.

Drug susceptibility analyses. Drug susceptibility was determined using a cell fusion assay as a measure of HIV-1 envelope susceptibility to attachment inhibitors. HIV-1 RNA was isolated from peripheral blood mononuclear cells from study subjects, and the *env* genes were amplified using published procedures (9). The PCR products were used to directly express the envelope protein using the TOPO expression kit (Invitrogen, Carlsbad, CA). The HIV-1 envelope-mediated fusion assay was performed using two populations of HeLa cells designated effector and target cells. The effector cells were prepared by cotransfecting 1.7×10^6 cells with 3 μg of linear envelope expression element and 3 μg of pTET-Off plasmid (BD Biosciences Pharmingen, San Diego, CA) using a Lipofectamine Plus kit (Invitrogen). After 4 h, the transfection mixture was removed, replaced with culture medium, and incubated overnight. HeLa cells expressing CD4, CXCR4, and CCR5 and containing an integrated copy of an inducible luciferase reporter gene were used as target cells (10). The effector and target cells were trypsinized, mixed at a ratio of 1:2, and seeded into a 96-well plate at 5×10^4/well in the presence of various concentrations of the test compound. After 12 to 18 h of incubation, luciferase activity was determined using a Steady-Glo Luciferase Assay System (Promega Corp., Madison, WI) and the EC_{50} was determined.

In vitro EC_{50}s were adjusted for protein binding prior to inclusion in the statistical analysis data sets. The protein binding-adjusted (PBA) EC_{50}s were computed by the following formula: PBA EC_{50} (ng/ml) = EC_{50} (nmol/liter) × 1/(1 − 0.95) × molecular weight of BMS-488043 (422.43)/1,000.

Statistical analyses. The primary assessment in the study was antiviral activity following 7.5 days of dosing of BMS-488043 as measured by the change in $\log_{10}$ HIV-1 RNA from the baseline to day 8 and the maximum decline in $\log_{10}$ HIV-1 RNA during the study period. A sample size of 12 subjects receiving a specified dose of BMS-488043 was calculated to provide 90% power to detect a decrease of 0.7 from the baseline in the mean $\log_{10}$ HIV-1 RNA level by day 8. The baseline was defined as the predose value on day 1 for all variables except $\log_{10}$ HIV-1 RNA, where the baseline was defined as the mean $\log_{10}$ HIV-1 RNA values before entry and predosing on day 1. Changes in $\log_{10}$ HIV-1 RNA from

the baseline were summarized by treatment and study day. The maximum decline from the baseline in $\log_{10}$ HIV-1 RNA levels over the entire study period was determined for each subject and summarized by treatment. Summary statistics for changes from the baseline in $CD4^+$ and $CD8^+$ T-cell counts were derived by treatment and study day. Summary statistics for pharmacokinetic parameters of BMS-488043 were tabulated by dose and study day. The association between the baseline susceptibility to BMS-488043 and antiviral activity was explored by scatter plots showing PBA EC_{50}s versus the observed change in $\log_{10}$ HIV-1 RNA from the baseline to day 8. The relationship was estimated using linear regression. The corresponding analysis was also performed for the maximum decline in $\log_{10}$ HIV-1 RNA from the baseline. The associations between pharmacokinetic measures of exposure to BMS-488043 and antiviral activity and between the ratio of drug exposure to the baseline viral susceptibility and antiviral activity were explored. Day 8 pharmacokinetic parameters, specifically, $AUC_{(TAU)}$, C_{max}, C_{trough}, and $C_{trough\ D6\text{-}8}$, were used as measures of exposure to BMS-488043, and the ratio of drug exposure to the baseline viral susceptibility was defined by the pharmacokinetic parameter divided by the baseline PBA EC_{50}. Change in viral susceptibility was assessed using the ratio of the EC_{50} of BMS-488043 on day 8 to that on day 1. All AEs recorded during the study were listed and tabulated by treatment, body system, and primary term. Vital signs and clinical laboratory test results were listed and summarized by treatment. Any significant physical examination findings and clinical laboratory results were listed. ECG recordings were evaluated by the investigator, and abnormalities, if present, were listed. Statistical analyses were performed using SAS/STAT Version 8.2 software (SAS Institute, Inc., Cary, NC).

RESULTS

Subject disposition and baseline characteristics. A total of 30 subjects were enrolled. All subjects completed treatment, with no doses missed and no subject discontinuing. Twelve subjects at each dose received BMS-488043, and three subjects at each dose received the placebo. The baseline characteristics of age, sex, race, and height were equally distributed among the treatment groups and BMS-488043 dose panels (Table 1); however, weight and body mass index appeared to be lower in the 800-mg dose group than in the 1,800-mg dose group and the placebo group. A higher percentage of subjects in the 1,800-mg dose group (67%) were antiretroviral treatment naïve than in the 800-mg dose group (42%) and the placebo group (50%). At the baseline, the mean HIV-1 RNA level was 4.55 $\log_{10}$ copies/ml and the median $CD4^+$ T-cell count was 368 cells/μl (Table 1).

TABLE 1. Baseline demographics and disease characteristics of the patients in this study

Characteristic	Placebo (n = 6)	BMS-488043 dose panel	
		800 mg (n = 12)	1,800 mg (n = 12)
Mean age, yr (SD)	41 (9)	40 (5)	39 (5)
No. (%) male	5 (83)	10 (83)	11 (92)
Race, n (%):			
White	4 (67)	8 (67)	8 (67)
Black	2 (33)	4 (33)	2 (17)
Other	0 (0)	0 (0)	2 (17)
Mean wt, kg (SD)	80.9 (9.3)	75.4 (14.6)	81.5 (10.5)
Mean ht, cm (SD)	173.7 (11.6)	175.6 (9.5)	176.6 (7.3)
Mean body mass index, kg/m^2 (SD)	27.0 (4.1)	24.4 (4.3)	26.2 (3.4)
No. (%) HIV antiretroviral naïve	3 (50)	5 (42)	8 (67)
No. (%) HIV antiretroviral experienced	3 (50)	7 (58)	4 (33)
Mean $\log_{10}$ HIV-1 RNA copies/ml (SD)	4.22 (0.49)	4.77 (0.71)	4.65 (0.42)
Median no. of $CD4^+$ T cells/μl (range)	417 (268–548)	368 (233–808)	318 (155–901)
Mean PBA EC_{50}, ng/ml (SD)	48.5 (32.2)[a]	165.2 (211.8)[b]	1,041 (2,142)[c]

[a] n = 4.
[b] n = 11.
[c] n = 11.

Antiviral activity. The mean change from the baseline in the $\log_{10}$ HIV-1 RNA level over time is shown in Fig. 1. Compared with the placebo, the plasma HIV-1 RNA level decreased from the baseline in a dose-dependent manner in subjects who received either the 800- or the 1,800-mg BMS-488043 dose. The mean HIV-1 RNA level decreases from the baseline (± the standard deviation) for the BMS-488043 800- and 1,800-mg dose groups on day 8 were 0.72 (± 0.51) and 0.96 (± 0.63) $\log_{10}$ copies/ml, respectively, compared with 0.02 (± 0.26) $\log_{10}$ copies/ml for the placebo group. The median maximum decreases in HIV-1 RNA from the baseline over the study period were 1.15 and 1.32 $\log_{10}$ copies/ml for the BMS-488043 800- and 1,800-mg dose groups, respectively, compared with 0.2 $\log_{10}$ copies/ml for the placebo group. Following initiation of treatment, plasma HIV-1 RNA continued to decline until approximately day 9 or for 1 day after the last dose of study medication. There were no remarkable differences in $CD4^+$ or $CD8^+$ T-cell counts between treatment groups (data not shown).

Pharmacokinetics. Following oral administration of a single dose of BMS-488043 or following twice-daily oral administration for 7.5 days, there were dose-related increases in the plasma BMS-488043 concentration for both the 800- and 1,800-mg doses (Table 2). These dose-related increases in the plasma BMS-488043 concentration were less than dose proportional. The C_{max} was reached around 4 h after dosing on both day 1 and day 8, with minimal accumulation of BMS-488043 (AI of 1.0 to 1.3) noted following multiple dose administrations, despite estimated half-lives of 15 to 17.7 h. Summary statistics of pharmacokinetic parameters are shown in Table 2.

Predictors of antiviral activity. The relationships between antiviral activity (represented by changes in the plasma HIV-1 RNA level from the baseline in $\log_{10}$ copies/ml following dosing with BMS-488043) and both viral susceptibility (as measured by *in vitro* PBA EC_{50} on day 1) and BMS-488043 pharmacokinetics were explored.

The baseline susceptibility to BMS-488043 showed a wide range of individual variability, with PBA EC_{50}s ranging from 27.71 to more than 1,000,000 ng/ml in the 800-mg dose group and from 5.83 to 6,868 ng/ml in the 1,800-mg dose group. A high EC_{50} at the baseline in both the 800- and 1,800-mg treatment groups was associated with a weaker antiviral response (Fig. 2). Conversely, a low EC_{50} at the baseline was associated with stronger antiviral response (Fig. 2). A similar relationship

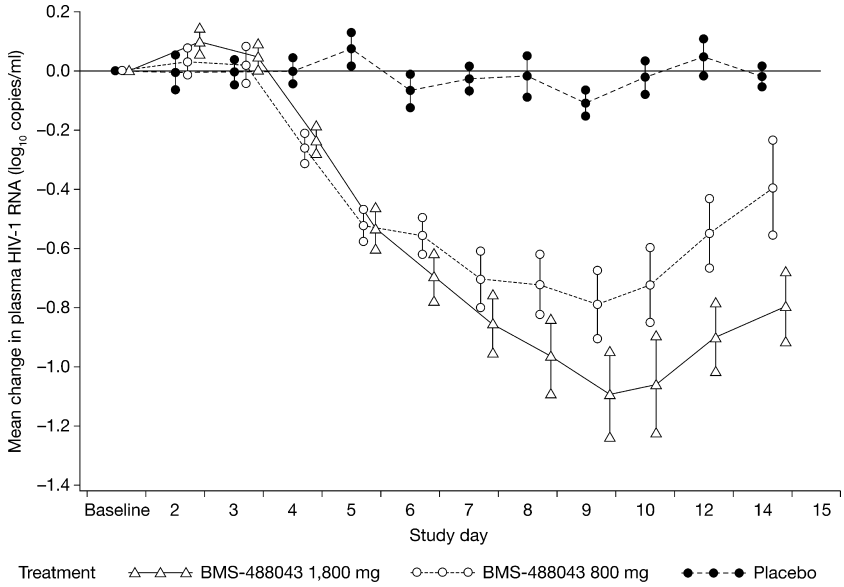

FIG. 1. Mean (standard error) changes in the $\log_{10}$ HIV-1 RNA level from the baseline following treatment with BMS-488043.

was observed regardless of whether antiviral activity was assessed by the day 8 change in HIV-1 RNA or by the maximum change in HIV-1 RNA.

No associations were noted between either the change on day 8 or the maximum change in $\log_{10}$ HIV-1 RNA levels from the baseline and the day 8 pharmacokinetic parameters $AUC_{(TAU)}$, C_{max}, C_{trough}, and geometric mean $C_{trough\ D6\text{-}8}$, which were used as different measures of exposure to the study drug.

However, an association was observed between BMS-488043 levels adjusted by the baseline BMS-488043 susceptibility, as estimated by EC_{50}s and antiviral activity. A scatter plot of viral load change from the baseline as a function of the ratio of geometric mean $C_{trough\ D6\text{-}8}$ to the baseline PBA EC_{50} is shown in Fig. 3. The estimated correlation coefficient was numerically higher when the change in plasma HIV-1 RNA from the baseline was correlated with the ratio of drug exposure to the baseline viral susceptibility ($r = 0.624$) than with the baseline viral susceptibility alone ($r = 0.519$; comparison of Fig. 3 with Fig. 2). Similar relationships were noted regardless of which pharmacokinetic parameter [$AUC_{(TAU)}$, C_{max}, C_{trough}, or $C_{trough\ D6\text{-}8}$] was used as a measure of drug exposure.

In summary, a low antiviral response in any given subject was predicted by a high baseline EC_{50} or a relatively low exposure to the study drug.

Viral resistance to BMS-488043. The change in susceptibility of viral isolates to BMS-488043 over the 7.5 days of treatment was measured as the ratio of the day 8 to the day 1 (predose) EC_{50}. BMS-488043 susceptibility assays displayed a variability of up to 10-fold when the same HIV-1 samples were retested. Based on this observation, only >10-fold decreases in BMS-488043 susceptibility were judged to represent convincing changes in susceptibility related to selection of BMS-488043 resistance. Consistent with this cutoff, HIV-1 from subjects who received the placebo experienced an up-to-6.16-fold decrease in BMS-488043 susceptibility.

Four subjects in the two dose groups developed resistance to BMS-488043, with resistance associated with a 33- to 344-fold decreased susceptibility by day 8. These subjects had a maximal reduction in HIV-1 RNA of no more than 0.81 $\log_{10}$ copies/ml (range, 0.38 to 0.81 $\log_{10}$ copies/ml), and their HIV-1 RNA decreases from the baseline to day 8 ranged between 0 and 0.81 $\log_{10}$ copies/ml. The baseline PBA EC_{50}s for these subjects (21.96, 41.90, 61.67, and 87.86 ng/ml) were well within the range of values observed for subjects who had a substantial virological response (Fig. 3) and who did not develop markedly decreased susceptibility to BMS-488043. Similarly, the ratio of the geometric mean $C_{trough\ D6\text{-}8}$ to the baseline PBA EC_{50} for

TABLE 2. Summary statistics for BMS-488043 pharmacokinetic parameters

Pharmacokinetic parameter	800 mg		1,800 mg	
	Day 1 ($n = 12$)	Day 8 ($n = 12$)	Day 1 ($n = 12$)	Day 8 ($n = 12$)
Geometric mean C_{max}, ng/ml (% CV)	2,353 (48.1)	2,833 (45.6)	3,712 (41.9)	4,109 (31.5)
Geometric mean $AUC_{(TAU)}$, ng · h/ml (% CV)	11,428 (54.2)	14,477 (39.6)	21,719 (33.2)	22,126 (31.9)
Geometric mean AI (% CV)		1.3 (100.7)		1.0 (27.4)
Median T_{max}, h (range)	4 (2–12)	4 (3–6)	4 (3–8)	4 (3–6)
$t_{1/2}$, h (SD)		15.0 (9.3)		17.7 (14.7)
Geometric mean C_{trough} , ng/ml (% CV)		354.8 (60.0)		738.0 (79.9)

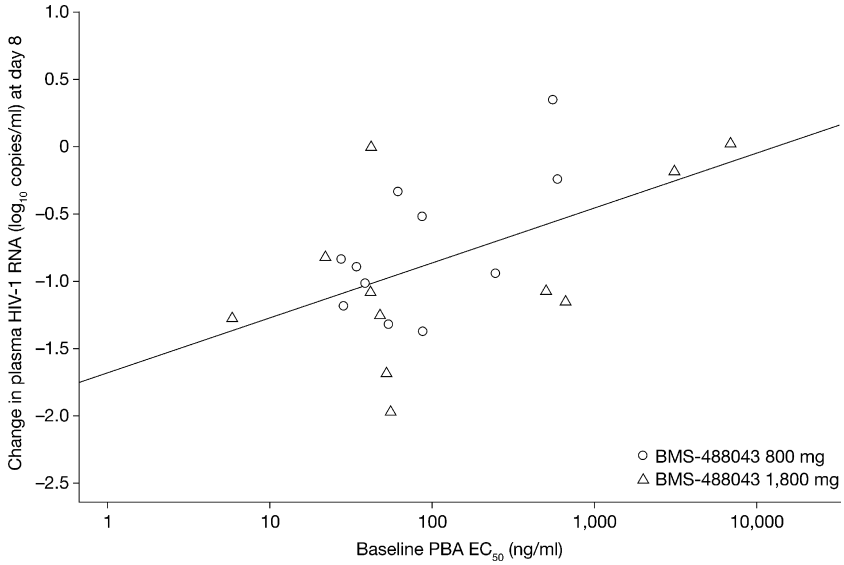

FIG. 2. Change in the $\log_{10}$ HIV-1 RNA level from the baseline at day 8 versus the baseline PBA EC_{50} with a fitted regression line. Fitted regression line: change from the baseline HIV-1 RNA level in $\log_{10}$ at day 8 = −1.67484 + 0.17666 ln (baseline PBA EC_{50}); 90% confidence interval for slope, 0.04095 to 0.31237; $P = 0.0133$; $R^2 = 0.2694$; $r = 0.519$. The value for one subject in the 800-mg BMS-488043 treatment arm was excluded from the graph because the baseline EC_{50} was above the quantification limit (PBA EC_{50}, >1,000,000 ng/ml). This subject experienced a change in the HIV-1 RNA level of −0.32 $\log_{10}$ at day 8; however, this change was within the expected assay and biological variability of clinical HIV-1 RNA measurements (~3-fold or ~0.5 $\log_{10}$). The values on the *x* axis are on a natural logarithm scale.

these four subjects was well within the range observed for subjects who did not develop resistance (data not shown).

Safety and tolerability. There were no SAEs or deaths following the administration of multiple oral doses of the study medication, and no AE led to study discontinuation. Overall, 31 AEs were reported in 13 (43%) of 30 subjects, including 28 AEs in 11 (46%) of 24 subjects who received BMS-488043 and 3 AEs in 2 (33%) of 6 subjects who received the placebo. Most of the reported AEs (28/31, 90%) were mild in intensity. The remaining three AEs were considered moderate in intensity and consisted of fatigue in a subject in the 800-mg group, an abscess in a subject in the 800-mg group, and diarrhea in a subject in the 1,800-mg group. The most common AE was fatigue, reported by five (21%) of the BMS-488043-treated subjects, all recipients of the 800-mg dose. There were no abnormalities in vital signs, physical examination, clinical lab-

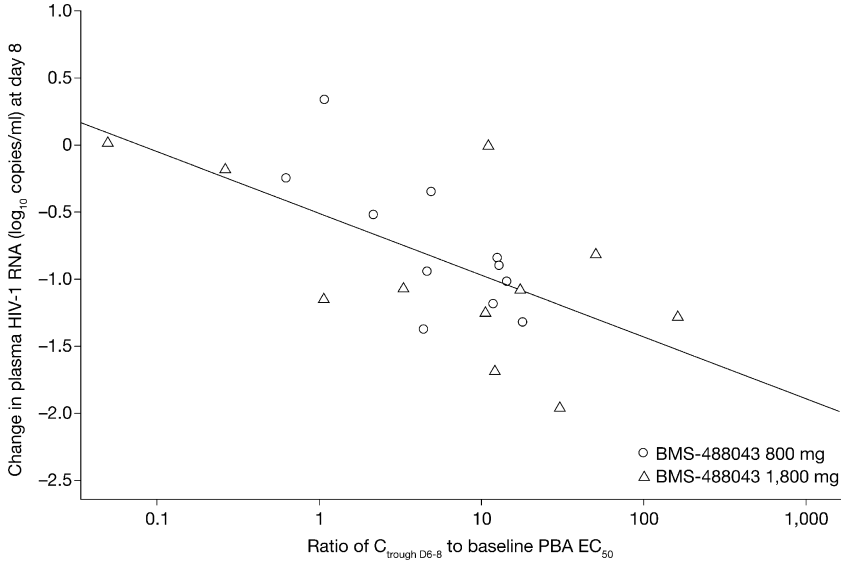

FIG. 3. Change in the $\log_{10}$ HIV-1 RNA level from the baseline at day 8 versus the ratio of the $C_{trough\ D6\text{-}8}$ to the baseline PBA EC_{50} with a fitted regression line. Fitted regression line: change from the baseline HIV-1 RNA level in $\log_{10}$ at day 8 = −0.50754 to 0.20065 ln (ratio of $C_{troughD6\text{-}8}$ to the baseline PBA EC_{50}); 90% confidence interval for slope, −0.31792 to −0.08338; $P = 0.0019$; $R^2 = 0.3891$; $r = 0.624$. The value for one subject in the 800-mg BMS-488043 treatment arm was excluded from the graph (see legend to Fig. 2). The values on the *x* axis are on a natural logarithm scale.

oratory tests, or ECGs that were considered clinically significant by the investigators.

DISCUSSION

BMS-488043 at a dose of 800 or 1,800 mg produced a reduction in plasma HIV-1 RNA from the baseline to day 8 of greater than 0.7 $\log_{10}$ copies/ml, providing proof of concept for this novel class of oral attachment inhibitors. Twice-daily administration of 800- and 1,800-mg doses for 7.5 days was generally safe and well tolerated, suggesting promising safety and tolerability of this class of antiretroviral agents. The pharmacokinetic results of this study indicate that the exposures to BMS-488043 were dose related but less than dose proportional, based on the 800- and 1,800-mg BMS-488043 doses administered with a high-fat meal. There was no apparent drug accumulation of the parent compound after twice-daily oral dosing for 7.5 days. The lack of accumulation of BMS-488043 in this study is more consistent with the shorter $t_{1/2}$ (11.1 h) noted following the administration of BMS-488043 as an oral solution (unpublished data) than the longer $t_{1/2}$ (15 to 17.7 h) reported in the present study. The $t_{1/2}$ reported in this study is most likely explained by absorption rate-limited pharmacokinetics as a result of the administration of BMS-488043 with a high-fat meal.

Although BMS-488043 achieved 10-fold higher plasma drug concentrations in humans than the prototype compound in this series, BMS-378806 (10, 15), a major limitation of BMS-488043 is its limited oral bioavailability. The requirement of a high-fat meal in order to achieve these exposures suggests that further optimization of this series of HIV-1 attachment inhibitors is preferred. Although improvements in drug formulation may have improved the bioavailability and mitigated limiting food effects, development of this compound was terminated to concentrate on newer-generation inhibitors. The profile targeted included better intrinsic potency and more favorable pharmacokinetic properties.

Of particular interest is the observed relationship between viral susceptibility to BMS-488043 and *in vivo* antiviral activity. This was evidenced by an association between a high mean EC_{50} at the baseline in both the 800- and 1,800-mg treatment groups and a weaker antiviral response. We were unable to discern an association between several BMS-488043 pharmacokinetic parameters, when not adjusted by the baseline viral drug susceptibility, and a virological response in this small study. This is likely due in part to the very wide range (10,000-fold) of individual variability in the BMS-488043 EC_{50}, in contrast to the much narrower range of individual variability of BMS-488043 pharmacokinetic parameters. The wide range of individual variability in the EC_{50} is likely to have obscured a relationship between drug exposure and virological response. Notably, BMS-488043 levels adjusted by the baseline viral BMS-488043 susceptibility were related to antiviral activity and resulted in a numerically stronger association with virological response than the baseline EC_{50} alone. This observation suggests that both viral susceptibility and drug levels are important determinants of viral response for this class.

Decreased susceptibility to BMS-488043 developed in four subjects who received BMS-488043 and none of those who received the placebo, providing further support of the direct antiviral mechanism of BMS-488043. The antiviral activity of BMS-488043 in these four subjects was modest. These subjects had virus susceptible to BMS-488043 at the baseline that developed resistance during the course of the monotherapy exposure. Resistance to BMS-488043 in cell culture has been demonstrated and was associated with specific mutations in gp120 (11). The underlying virological and/or biological mechanisms that allowed the rapid development of resistance in the four subjects who developed drug resistance during BMS-488043 administration in our study are being investigated. However, the limited number of subjects precludes a robust analysis of predictors of resistance selection in this small study.

These results suggest the need to decrease the intrinsic variability of antiviral activity of future attachment inhibitors in this series while maintaining a favorable pharmacokinetic, safety, and tolerability profile. Newer attachment inhibitors with improved bioavailability and EC_{50}s have been developed, and one of them, BMS-663068, is currently in early clinical development (ClinicalTrials.gov no. NCT01009814). Although BMS-488043 is no longer in clinical development, its potent anti-HIV activity, unique mode of action, and promising pharmacokinetic and safety profile warrant further development of this novel class of oral attachment inhibitors that target HIV-1 gp120 and prevent the binding of virus to $CD4^+$ lymphocytes in HIV-1-infected persons.

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REFERENCES

1. **Alkhatib, G.** 2009. The biology of CCR5 and CXCR4. Curr. Opin. HIV AIDS **4:**96–103.
2. **Dau, B., and M. Holodniy.** 2009. Novel targets for antiretroviral therapy: clinical progress to date. Drugs **69:**31–50.
3. **Esté, J. A., and A. Telenti.** 2007. HIV entry inhibitors. Lancet **370**(9581)**:** 81–88.
4. **Fakes, M. G., et al.** 2009. Enhancement of oral bioavailability of an HIV-attachment inhibitor by nanosizing and amorphous formulation approaches. Int. J. Pharm. **370:**167–174.
5. **Guo, Q., et al.** 2003. Biochemical and genetic characterizations of a novel human immunodeficiency virus type 1 inhibitor that blocks gp120-CD4 interactions. J. Virol. **77:**10528–10536.
6. **Hanna, G., et al.** 2004. Safety, tolerability and pharmacokinetics (PK) of a novel, small-molecule HIV-1 attachment inhibitor, BMS-488043, after single and multiple oral doses in healthy subjects, poster 535, p. 257. Program Abstr. 11th Conf. Retrovir. Oppor. Infect. 2004, San Francisco, CA.
7. **Ho, H. T., et al.** 2006. Envelope conformational changes induced by human immunodeficiency virus type 1 attachment inhibitors prevent CD4 binding and downstream entry events. J. Virol. **80:**4017–4025.

8. **Kuritzkes, D. R.** 2009. HIV-1 entry inhibitors: an overview. Curr. Opin. HIV AIDS **4:**82–87.
9. **Li, M., et al.** 2005. Human immunodeficiency virus type 1 *env* clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. J. Virol. **79:**10108–10125.
10. **Lin, P. F., et al.** 2003. A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding. Proc. Natl. Acad. Sci. U. S. A. **100:**11013–11018.
11. **Lin, P. F., et al.** 2004. Characterization of a small molecule HIV-1 attachment inhibitor BMS-488043: virology, resistance, and mechanism of action, poster 534, p. 256. Program Abstr. 11th Conf. Retrovir. Oppor. Infect. 2004, San Francisco, CA.
12. **Marr, P., and S. Walmsley.** 2008. Reassessment of enfuvirtide's role in the management of HIV-1 infection. Expert Opin. Pharmacother. **9:**2349–2362.
13. **Tilton, J. C., and R. W. Doms.** 2010. Entry inhibitors in the treatment of HIV-1 infection. Antiviral Res. **85:**91–100.
14. **Wang, T., et al.** 2009. Inhibitors of human immunodeficiency virus type 1 (HIV-1) attachment. 5. An evolution from indole to azaindoles leading to the discovery of 1-(4-benzoylpiperazin-1-yl)-2-(4,7-dimethoxy-1H-pyrrolo[2,3-c]pyridin-3-yl)ethane-1,2-dione (BMS-488043), a drug candidate that demonstrates antiviral activity in HIV-1-infected subjects. J. Med. Chem. **52:**7778–7787.
15. **Wang, T., et al.** 2003. Discovery of 4-benzoyl-1-[(4-methoxy-1H-pyrrolo[2,3-b]pyridin-3-yl)oxoacetyl]-2-(R)-methylpiperazine (BMS-378806): a novel HIV-1 attachment inhibitor that interferes with CD4-gp120 interactions. J. Med. Chem. **46:**4236–4239.
16. **Xue, Y. J., J. H. Yan, M. Arnold, D. Grasela, and S. Unger.** 2007. Quantitative determination of BMS-378806 in human plasma and urine by high-performance liquid chromatography/tandem mass spectrometry. J. Sep. Sci. **30:**1267–1275.
17. **Yang, Z., et al.** 2010. Utilization of in vitro Caco-2 permeability and liver microsomal half-life screens in discovering BMS-488043, a novel HIV-1 attachment inhibitor with improved pharmacokinetic properties. J. Pharm. Sci. **99:**2135–2152.