Prediction of Virological Response and Assessment of Resistance Emergence to the HIV-1 Attachment Inhibitor BMS-626529 During 8-Day Monotherapy With Its Prodrug BMS-663068

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Background: BMS-663068 is the phosphonooxymethyl prodrug of BMS-626529, a small-molecule attachment inhibitor that targets the HIV-1 envelope glycoprotein gp120 preventing it from binding to $CD4^+$ T cells. In vitro investigations have demonstrated considerable variation in susceptibility of different HIV-1 isolates to BMS-626529. BMS-663068 monotherapy in HIV-1–infected subjects produced a mean maximum change from baseline of -1.64 $\log_{10}$ copies per milliliter, but the response was variable.

Methods: In this analysis, baseline and day 8 samples were analyzed for susceptibility to BMS-626529 and the presence of known HIV-1 attachment inhibitor resistance mutations. In addition, predictors of virological response (maximal HIV-1 RNA decline ≥ 1 $\log_{10}$ copies per milliliter) and resistance selection were investigated.

Results: The only factor associated with reduced virological response was low baseline susceptibility to BMS-626529. There was no apparent relationship between virological response and baseline treatment experience, coreceptor tropism, plasma HIV-1 RNA level, or $CD4^+$ T-cell count. Examination of all positions with known BMS-626529 resistance mutations based on in vitro selection studies showed that gp120 M426L was the primary substitution most clearly associated with nonresponse to BMS-663068. There was minimal change in susceptibility to BMS-626529 over the course of the study and no clear evidence of emergence of a known HIV-1 attachment inhibitor resistance mutation in the majority of subjects as measured by standard population-based phenotypic and genotypic approaches.

Conclusions: Nonresponse to BMS-663068 was associated with low baseline susceptibility to BMS-626529 and the presence of M426L. In this short-term trial, there was minimal evidence of selection for BMS-626529 high-level resistance over 8 days of monotherapy with BMS-663068 by population-based approaches.

Key Words: BMS-626529, BMS-663068, attachment inhibitor, virological response, resistance

(*J Acquir Immune Defic Syndr* 2013;64:7–15)

INTRODUCTION

There is a continuing need for the development of novel antiretroviral drugs and regimens to address the tolerability and long-term safety concerns associated with current treatment options, the immune dysfunction induced by HIV infection, and the emergence of antiretroviral drug resistance.[1,2]

HIV-1 attachment inhibitors (AIs) represent a novel class of small-molecule antiretroviral agents that bind to the HIV-1 envelope glycoprotein, gp120, and selectively inhibit the interaction between the virus and its host receptor, CD4, thereby preventing viral entry into the host cells.[3] Proof-of-concept for this class was achieved in an 8-day placebo-controlled monotherapy trial of the progenitor AI BMS-488043.[4] In this study, BMS-488043 demonstrated potent antiviral activity; however, there was significant variability in individual half-maximal effective concentration (EC_{50}) values.[5] Four of the 30 enrolled subjects had baseline EC_{50} >200 nM, and 4 of the 24 subjects who received BMS-488043 had emergent phenotypic resistance (EC_{50} >10-fold higher than baseline) over the course of the study. Five gp120 mutations were identified that were associated with BMS-488043 resistance (V68A, L116I, S375I/N, and M426L).[5]

Efforts to increase the inhibitory potency of the AI class against specific HIV-1 isolates resulted in the discovery of

Received for publication December 18, 2012; accepted April 12, 2013.

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Supported by Bristol-Myers Squibb.

Presented in part at the 19th Annual Conference on Retroviruses and Opportunistic Infections, March 5–8, 2012, Seattle, WA (poster 725), and the International Workshop on HIV & Hepatitis Virus Drug Resistance, June 5–9, 2012, Sitges, Spain (poster 6).

N.R., C.H., M.D.H., M.L., M.W-R., M.K., and G.J.H. are employees of and shareholders in Bristol-Myers Squibb. J.W. is an employee of Monogram Biosciences/Laboratory Corporation of America, who received fees for some of the work included within this publication, and is a shareholder in the Laboratory Corporation of America.

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BMS-626529.[6] Low solubility and poor intrinsic dissolution properties of BMS-626529 were addressed through development of the phosphonooxymethyl prodrug BMS-663068, which has been examined in a proof-of-concept study.[7]

In vitro investigations have demonstrated considerable variation in susceptibility of HIV-1 envelopes to BMS-626529. The majority of viral isolates were susceptible, with EC_{50} values <10 nM; however, susceptibility varied by >6 $\log_{10}$, with EC_{50} values for the most susceptible viruses in the low pM range.[6] BMS-626529 displayed activity against almost all subtypes tested, with subtype B appearing to be most susceptible, followed by subtype C and then subtype A. Only subtype AE and possibly group O were not susceptible to BMS-626529 in vitro.[6] Susceptibility was observed for envelopes with CCR5, CXCR4, or dual tropism.[6]

In an 8-day monotherapy study of treatment-naive and treatment-experienced HIV-1–infected subjects, all of whom were infected with subtype B virus, BMS-626529, administered as BMS-663068, effected significant reductions in plasma HIV-1 RNA, with an average maximum change from baseline of -1.64 $\log_{10}$ copies per milliliter (range, -2.395 to $+0.039$ $\log_{10}$ copies per milliliter).[7] Previous analyses from this study showed that plasma concentrations of BMS-626529 were not associated with antiviral response, whereas baseline half-maximal inhibitory concentration (IC_{50}) values were correlated with virological response.[7]

The present analysis examined the relationship between virological response and baseline factors in the 8-day monotherapy study of BMS-663068. Susceptibility of envelopes to BMS-626529 and the presence of substitutions shown to affect susceptibility to BMS-626529 in vitro were analyzed. In addition, viral populations were analyzed after treatment to determine whether short-term monotherapy selected for reductions in susceptibility or for the emergence of any known resistance-associated substitutions.

METHODS

Study Design and Subjects

This was a randomized, open-label, multiple-dose parallel study, which has previously been described in detail.[7] Five dose regimens of BMS-663068 were evaluated for 8 days: group 1, 600 mg BMS-663068 plus 100 mg ritonavir every 12 hours (Q12H); group 2, 1200 mg BMS-663068 plus 100 mg ritonavir every bedtime; group 3, 1200 mg BMS-663068 plus 100 mg ritonavir Q12H; group 4, 1200 mg BMS-663068 Q12H plus 100 mg ritonavir every morning; group 5, 1200 mg BMS-663068 Q12H. Eligible subjects were adults (aged $\geq$18 years) infected with subtype B HIV-1 who had plasma HIV-1 RNA levels $\geq$5000 copies per milliliter (assessed using a Roche COBAS Amplicor Analyzer; Roche Diagnostics, Indianapolis, IN) and $CD4^+$ T-cell counts $\geq$200 cells per microliter. Viral tropism was determined using the Trofile assay (Monogram Biosciences, San Francisco, CA). Subjects could be antiretroviral treatment naive (defined as not having received therapy for $\geq$1 week) or experienced; however, all antiretroviral therapy must have been discontinued for at least 8 weeks before participation in the study. For the purpose of the analyses reported here, we defined a virological responder as a subject with a maximal decline in plasma HIV-1 RNA of ≥ 1 $\log_{10}$ copies per milliliter and a nonresponder as a subject with a maximal decline in plasma HIV-1 RNA of <1 $\log_{10}$ copies per milliliter.

All subjects provided written informed consent before participation in the study. 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 the study site.

Susceptibility Assay

The susceptibility of subjects' viral quasispecies at days 1 (baseline) and 8 was assessed from plasma using the PhenoSense Entry assay (Monogram Biosciences). Originally, the highest concentration of BMS-626529 tested in the assay was 100 nM.[7] As 7 subjects had IC_{50} values above this concentration, samples were retested with a maximum concentration of BMS-626529 of 10 μM. To minimize interassay variability, subjects' IC_{50} values were normalized to that of a control virus tested within the same analytical batch, to obtain fold change IC_{50} (FC-IC_{50}). The control virus used for normalization was DUAL, derived from a drug-naive reference virus (92HT594) that can efficiently infect cells expressing the CD4 receptor and either the CXCR4 or CCR5 coreceptor molecules on their surface (IC_{50} range: 0.42–1.0 nM; mean: 0.74 nM) (HIV-1 92HT594 was obtained from Dr Neal Halsey and the AIDS Research and Reference Reagent Program, Division of AIDS [DAIDS], NIAID, NIH). Target cells were U87 cells engineered to express CD4, CXCR4, and CCR5 on their surface (obtained through the AIDS Research and Reference Reagent Program, DAIDS).[8] Assay validation experiments (data on file at Monogram Biosciences) demonstrate that $>$95% of replicate measures of FC-IC_{50} in the PhenoSense Entry assay are within 3-fold of each other and that approximately 90% are within 2-fold of each other.

Population *env* (gp160) Sequencing

Population sequencing of baseline and day 8 samples was performed using GeneSeq Env (Monogram Biosciences). The GeneSeq Env assay analyzes the entire HIV gp160 envelope glycoprotein gene (*env*). Indeterminate regions are common in HIV *env* population sequences, primarily due to the presence of sequence length heterogeneity (insertion/deletions), especially in the variable regions encoding gp120. Multiple alignment of the subjects' *env* sequences and HXB2 reference amino acid sequence was performed using ClustalX.[9] Nucleotide sequences were aligned to the HXB2 reference amino acid sequence using the FrameSearch algorithm[10] for assigning nucleotides to codons. Where the nucleotide sequence had mixture calls, translations were made by expanding all possibilities within the codon. If there was only one possible translation, that amino acid letter was assigned; if there were 2 possibilities, both amino acid letters were assigned. In the event of 3 or more possible translations, or if the sequence was unreadable due to insertions/deletions, an X was assigned for the translation at that codon. In cases where an X was assigned at baseline and the day 8 sequence was the same as the HXB2 reference

sequence, the amino acid present within the HXB2 reference sequence was also assigned to the baseline sequence. The nucleotide sequences have been deposited in GenBank.

Positions with known AI resistance mutations based on previous in vitro selection studies were examined manually for association with virological response. These included L116P, A204D, M426L, M434I, M475I, and V506M, which have all been identified as substitutions associated with in vitro resistance to BMS-626529.[11] In addition, substitutions at positions 68, 185, 255, 281, 375, 423, 506, 595, and 655 have been associated with in vitro resistance to other AI analogs[5,11–13] (unpublished data, Bristol-Myers Squibb, 2011). Specifically, position 375 was included in this study because it is an important position for in vivo resistance to BMS-488043, the progenitor AI.[5]

Statistical Analyses

An automatic scan of all positions in the multiple sequence alignment was performed using the Fisher exact test, a 2-tailed mid-*P* variant.[14] For each position in the alignment, the following counts were made:

- Number of nonresponders same as HXB2 here
- Number of nonresponders different from HXB2 here
- Number of responders same as HXB2 here
- Number of responders different from HXB2 here

From these counts, a *P* value was computed for the hypothesis that variability at this position was significantly different in either direction for responder versus nonresponder subjects (ie, a 2-tailed hypothesis test). Each of the 10 positions in the sequence alignment having the smallest *P* values was manually examined for association with virological response. For some positions, it was not possible to compute a *P* value, either because all subjects in both groups were the same as HXB2 at that position or because no subject in either group was the same as HXB2. It was possible to perform a Fisher exact test calculation at 616 positions, and adjustment for multiple comparisons was performed using Bonferroni correction. It should be noted that with the limited number of subjects in this study, statistical power to detect either rare mutations or mutations with a small effect on drug resistance was lacking.

RESULTS

Efficacy

Fifty subjects were randomly assigned to treatment, and 48 subjects completed the study (2 subjects were withdrawn because they did not meet the inclusion criteria; both were infected with non-subtype B HIV-1). Baseline demographics and disease characteristics were generally well balanced between the regimen groups.[7] Of the 48 subjects who completed the study, there were 42 responders ($\geq$1.0 $\log_{10}$ copies per milliliter decline in plasma HIV-1 RNA) and 6 nonresponders.

Predictors of Virological Response

Susceptibility to BMS-626529 was obtained for 46 subjects at baseline; IC_{50} values could not be obtained for 2 subjects (who were responders) because of assay failure. All 6 nonresponders had a baseline IC_{50} >100 nM.[7] Furthermore, all nonresponders had high baseline FC-IC_{50} (>100). Figure 1A shows individual baseline FC-IC_{50} values plotted against maximum reduction in plasma HIV-1 RNA levels. These data indicate that there was a correlation between the 2 variables, with nonresponse to BMS-663068 being predicted by high baseline BMS-626529 FC-IC_{50}. It should be noted that 1 individual (subject 5) demonstrated a robust maximum decline in plasma HIV-1 RNA of -1.61 $\log_{10}$ copies per milliliter, despite having a very high baseline IC_{50} of 5.29 μM and an FC-IC_{50} of 6136.5; the reasons for this are not currently understood.

The relationship between virological response and selected baseline parameters, including treatment experience, coreceptor tropism, plasma HIV-1 RNA level, and $CD4^+$ T-cell count, is shown in Figures 1B–E. These data show that none of these baseline variables were predictive of response.

To evaluate whether there was an association between BMS-626529 resistance–associated envelope substitutions and virological response, multiple sequence alignment of subjects' envelope genes and the HXB2 amino acid sequence was performed. Owing to the polymorphic nature of gp160 and the presence of sequence length heterogeneity, complete *env* sequences could not be obtained by population sequencing for all subject samples. As a result, the sequences from certain populations contained stretches of unreadable or ambiguous sequences as indicated in Table 1. Previous in vitro studies have identified a series of substitutions at 7 positions that may encode for reduced susceptibility to BMS-626529.[11] Examination of all these markers showed that M426L was the only substitution that appeared to have an association with nonresponse to BMS-626529 (Table 1), as 4 out of the 6 nonresponders had this substitution. A fifth nonresponder, whose baseline sequence was unreadable (X) at position 426, harbored L426 on day 8, which may have preexisted in this subject at baseline. However, M426L was also present in 2 responders, indicating that this substitution does not preclude response to BMS-663068. One of the responders, who harbored M426L (subject 21), had an intermediate baseline IC_{50} of 16.4 nM (FC-IC_{50}: 39.0) and a correspondingly moderate maximal decline in plasma HIV-1 RNA of -1.08 $\log_{10}$ copies per milliliter, whereas the other responder to harbor M426L (subject 5) demonstrated a robust maximal decline in plasma HIV-1 RNA of -1.61 $\log_{10}$ copies per milliliter, although this subject also had a very high baseline IC_{50} of 5.29 μM (FC-IC_{50}: 6136.5). The only nonresponder without the M426L substitution at baseline (subject 41; IC_{50}: 1.36 μM; FC-IC_{50}: 1433.1) possessed M434I (a BMS-626529 resistance–associated mutation) and S375M substitutions, the latter of which is at a position of known importance in clinical resistance to the previous AI, BMS-448043.[5] The smallest Fisher exact test *P* value for association with virological response, determined by an automatic scan of all *env* positions in the multiple sequence alignment, was 0.0033 at position 426, but after adjusting for the 616 multiple comparisons (Bonferroni correction), this did not achieve statistical significance. Of the remaining 9 positions with smallest *P* values, none were at positions of known BMS-626529 resistance

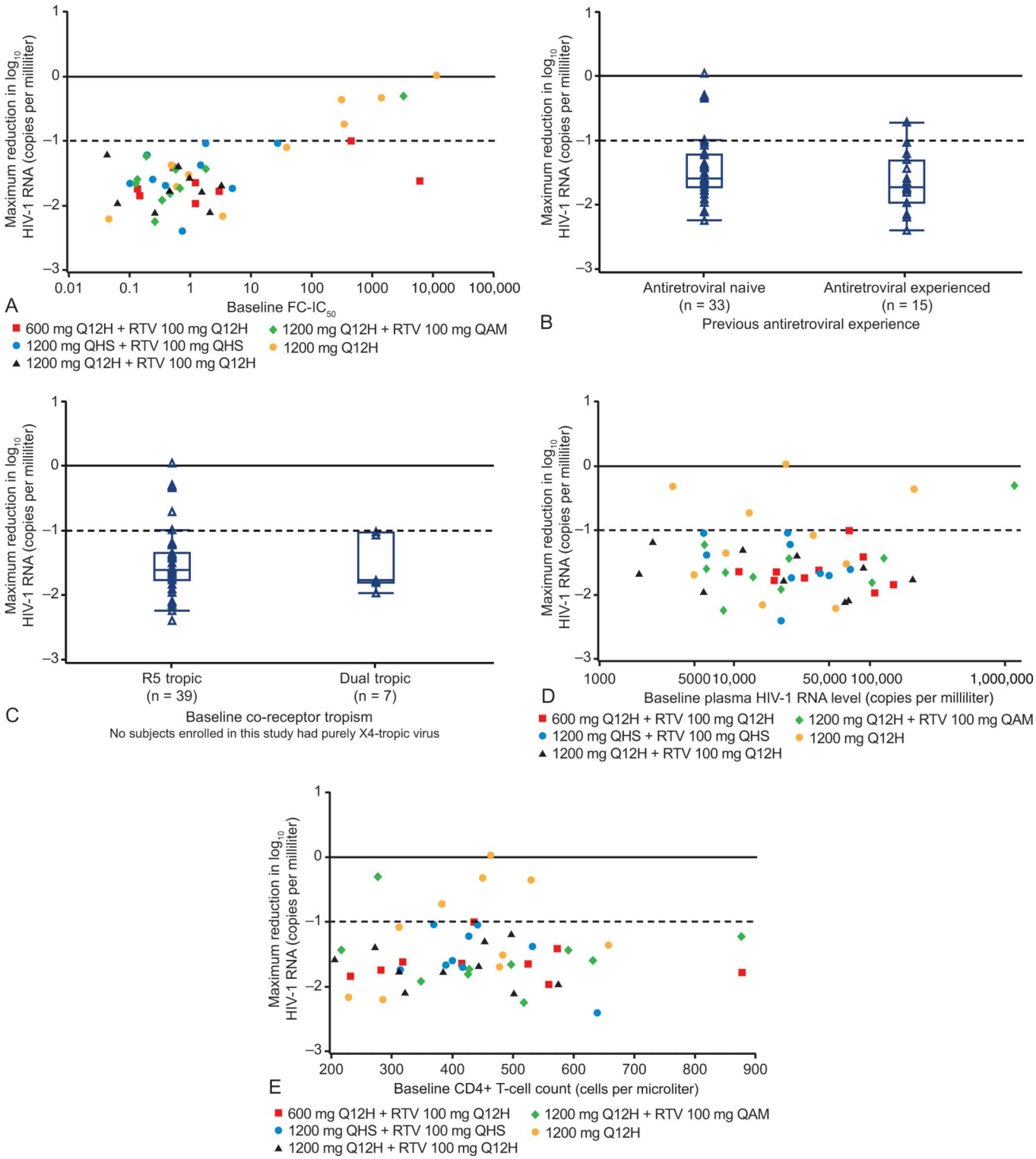

FIGURE 1. Relationship between baseline parameters and virological response. FC-IC_{50} (A), treatment experience (B), coreceptor tropism (C), plasma HIV-1 RNA level (D), and $CD4^+$ T-cell count (E). QAM, every morning; QHS, every bedtime; Q12H, every 12 hours; RTV, ritonavir.

TABLE 1. Association of Baseline *env* Genotypes With Response

B-consensus	AI-Selected Substitution With High Resistance to BMS-626529	HXB2 Position	Nonresponders* 1	16	41	48	54	70	Responders† 3	4	5	6
Maximum change in plasma HIV-1 RNA (log_{10})			−0.72	−0.30	−0.31	−0.04	−0.99	−0.35	−1.73	−1.21	−1.61	−1.84
L	*P*	116	•	•	•	•	•	•	•	•	•	•
A	*D/V*	204	•	•	•	•	•	•	•	•	•	•
S	*I/N*	375	•	•	M	I/T	•	•	•	•	•	T
M	*L*	426	*L*	*L*	•	*L*	*X*	*L*	R	R	*L*	R
M	*I*	434	•	•	*I*	•	*X*	•	•	•	•	•
M	*I*	475	•	*I*	•	•	•	•	•	•	•	•
V	*M*	506	•	•	•	•	•	•	•	•	•	•

B-consensus	AI-Selected Substitution With High Resistance to BMS-626529	HXB2 Position	Responders† 7	8	9	10	12	13	17	19	21	22
Maximum change in plasma HIV-1 RNA (log_{10})			−1.77	−1.35	−1.60	−1.40	−1.03	−1.81	−1.97	−1.58	−1.08	−2.20
L	*P*	116	–	•	–	•	•	•	•	•	•	•
A	*D/V*	204	–	•	•	•	•	•	•	•	•	S
S	*I/N*	375	–	•	•	•	H	•	T	T	•	•
M	*L*	426	–	•	X	X	R	R	•	X	*L*	•
M	*I*	434	–	•	X	X	•	•	•	X	•	•
M	*I*	475	–	•	X	•	•	•	•	•	•	•
V	*M*	506	–	•	X	•	•	•	•	•	•	•

B-consensus	AI-Selected Substitution With High Resistance to BMS-626529	HXB2 Position	Responders† 27	28	29	30	31	34	35	36	38	40	42	43	45
Maximum change in plasma HIV-1 RNA (log_{10})			−1.19	−1.43	−1.66	−1.66	−2.39	−1.69	−1.64	−1.74	−1.03	−2.15	−2.10	−1.69	−1.91
L	*P*	116	•	•	•	•	•	•	•	•	•	•	•	•	•
A	*D/V*	204	•	•	•	•	•	•	A/T	•	•	•	•	•	•
S	*I/N*	375	•	T	•	X	•	•	•	•	T	T	•	T	•
M	*L*	426	•	X	•	X	X	•	•	•	•	X	•	X	X
M	*I*	434	•	X	•	X	X	•	*I*	•	•	X	•	X	•
M	*I*	475	•	•	•	•	•	•	•	•	•	•	•	•	•
V	*M*	506	•	•	•	•	•	•	•	•	•	•	•	•	•

B-consensus	AI-Selected Substitution With High Resistance to BMS-626529	HXB2 Position	Responders† 46	47	49	53	56	57	59	61	64	66	67	68	73
Maximum change in plasma HIV-1 RNA (log_{10})			−1.78	−1.59	−2.25	−1.41	−1.23	−1.37	−1.97	−2.11	−1.51	−1.73	−1.78	−1.68	−1.43
L	*P*	116	•	•	•	•	•	•	•	•	•	•	•	•	•
A	*D/V*	204	•	•	•	•	•	•	•	•	•	•	•	•	•
S	*I/N*	375	•	•	•	T	•	T	•	T	•	T	T	T	•
M	*L*	426	X	•	•	•	•	•	•	R	•	•	•	•	•
M	*I*	434	•	•	•	•	•	•	•	*I*/M	•	•	*I*	•	•
M	*I*	475	•	•	•	•	•	•	•	•	•	•	•	•	•
V	*M*	506	•	•	•	•	•	•	•	•	•	•	•	•	•

–, sequence unavailable. X represents an unreadable/ambiguous amino acid and other letters represent single letter amino acid abbreviations. Italics represent AI-selected substitutions with high resistance to BMS-626529.

*Nonresponder = maximum decline in plasma HIV-1 RNA <1 log_{10} copies per milliliter.

†Responder = maximum decline in plasma HIV-1 RNA ≥ 1 log_{10} copies per milliliter.

substitutions (see **Table S1**, **Supplemental Digital Content**, http://links.lww.com/QAI/A427).

Changes Between Baseline and Day 8

Susceptibility and sequence data at both baseline and day 8 were available for 38 subjects [data were not available for 10 subjects due to assay failure at baseline (2 subjects) or day 8 (8 subjects); the latter failures were most likely due to low viral load]. Changes in FC-IC_{50} day 8/day 1 were minimal, generally well within the assay variability range for FC-IC_{50} in this assay (2- to 3-fold), and similar across treatment groups (Fig. 2). There was a suggestion of an increase in FC-IC_{50} at day 8 in all treatment groups; however, the magnitude of these increases was relatively small.

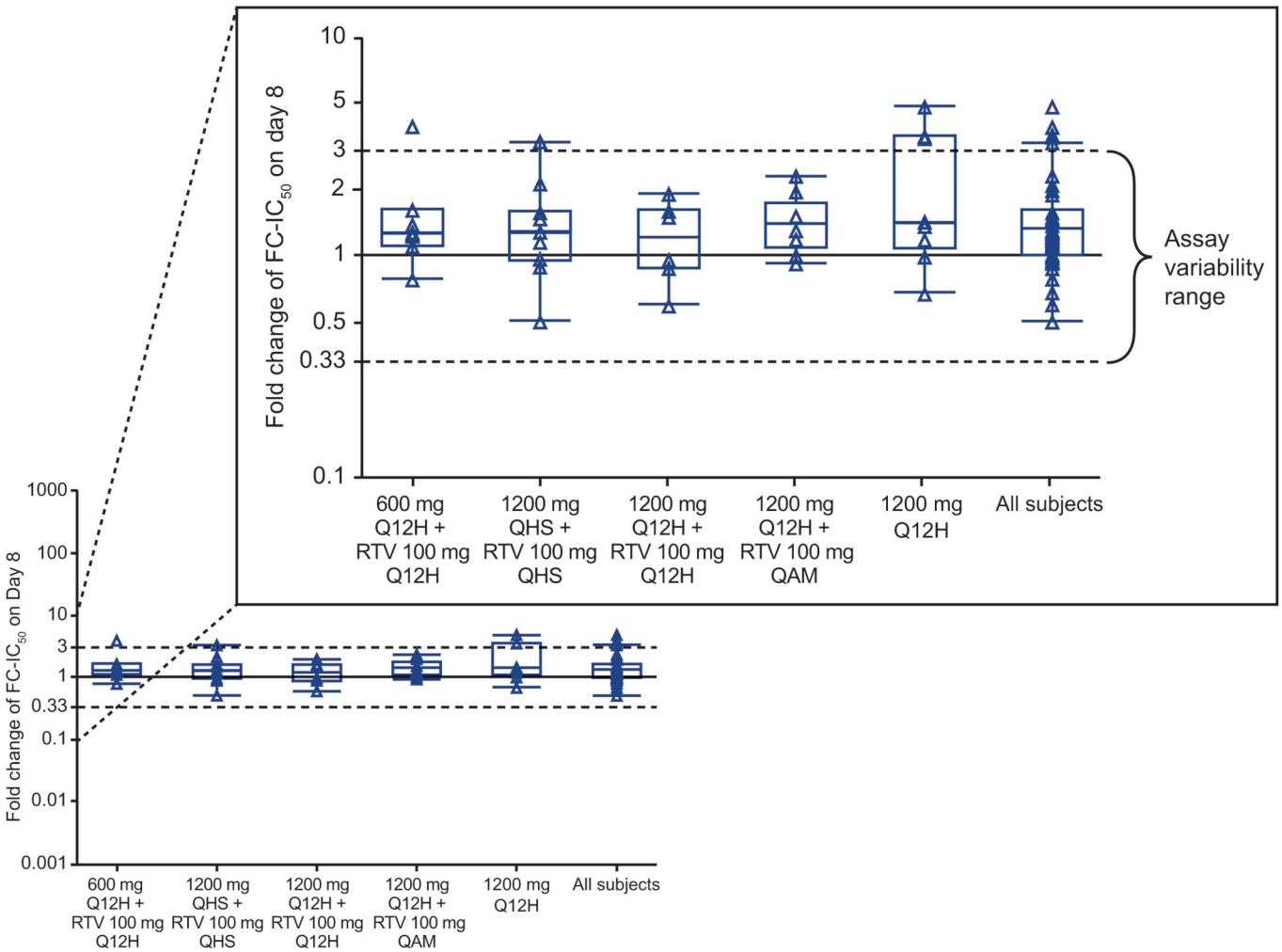

FIGURE 2. Distribution of FC-IC_{50} day 8/day 1 values. QAM, every morning; QHS, every bedtime; Q12H, every 12 hours; RTV, ritonavir.

The median FC-IC_{50} day 8/day 1 was 1.33 for all subjects combined, with the majority of subjects (33 of 38) demonstrating changes in FC-IC_{50} from baseline to day 8 of <3-fold. Five subjects (3 nonresponders and 2 responders) had increases in FC-IC_{50} only marginally higher than the assay variability range, with none >5-fold. Examination of these 5 subjects showed that the 3 nonresponders already had high IC_{50} (FC-IC_{50}) values [1357 nM (1433), 270 nM (314), and

TABLE 2. Changes in *env* Genotype of Subjects With >3-fold Increases in FC-IC_{50}

Subject	Maximum Decline in log_{10} Plasma HIV-1 RNA	Baseline IC_{50} (nM)	Baseline FC-IC_{50}	Fold Change of FC-IC_{50} on Day 8	HXB2 Position B-consensus AI-Selected Substitution	116 L P	204 A D/V	375 S N/I	426 M L	434 M I	475 M I	506 V M
30	−1.66	0.043	0.10	3.28	Baseline	•	•	X	X	X	•	•
					Day 8	•	X	X	X	X	•	•
41	−0.31	1357	1433	3.5	Baseline	•	•	M	•	*I*	•	•
					Day 8	•	•	M	•	*I*	•	•
54	−0.99	386	448	3.87	Baseline	•	•	•	X	X	•	•
					Day 8	•	•	•	*L*	*I*	•	•
64	−1.51	0.949	0.95	4.77	Baseline	•	•	•	•	•	•	•
					Day 8	•	•	•	*L*/M	•	•	•
70	−0.35	270	314	3.57	Baseline	•	•	•	*L*	•	•	•
					Day 8	•	•	•	*L*	•	•	•

X represents an unreadable/ambiguous amino acid and other letters represent single letter amino acid abbreviations. Italics represent AI-selected substitutions with high resistance to BMS-626529.

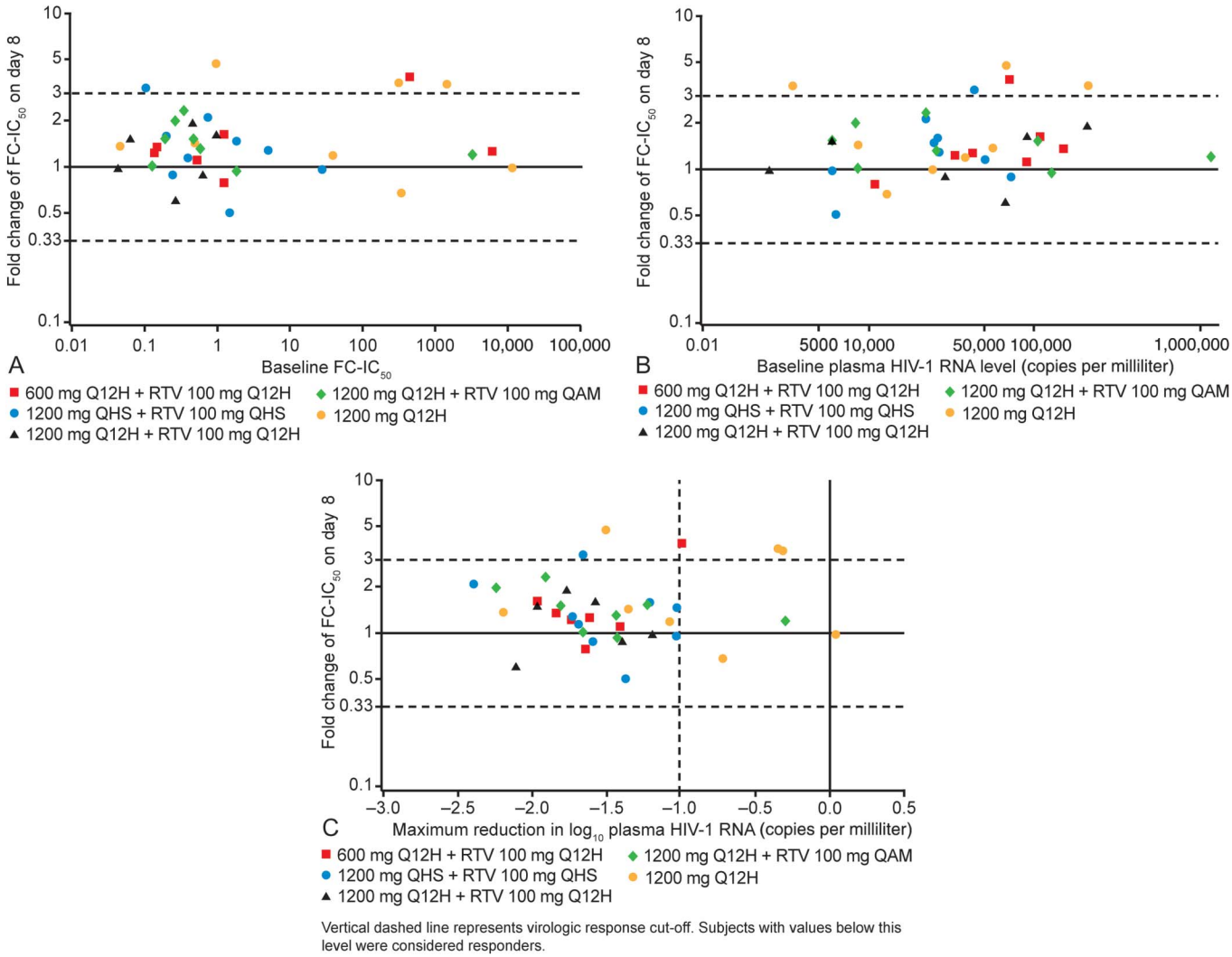

FIGURE 3. Relationship between FC-IC_{50} day 8/day 1 and baseline FC-IC_{50} (A), baseline plasma HIV-1 RNA level (B), and maximum reduction in plasma HIV-1 RNA (C). QAM, every morning; QHS, every bedtime; Q12H, every 12 hours; RTV, ritonavir.

386 nM (448)] at baseline, whereas the 2 responders continued to exhibit low IC_{50}s (FC-IC_{50}s) on day 8 [0.2 nM (0.337) and 3 nM (4.54)].

Analysis of the *env* sequence from the 5 subjects with changes in FC-IC_{50} from baseline to day 8 of >3-fold showed that 1 of these 5 subjects (subject 64; responder) had changes at position 426 that may represent enrichment of envelopes with L426 or suppression of envelopes with M426 (Table 2). Another subject (subject 54; nonresponder) who had changes in FC-IC_{50} from baseline to day 8 of >3-fold had an unreadable sequence at position 426 at baseline but harbored L426 on day 8. It is unclear whether the L426 substitution in this subject arose over the course of therapy or preexisted at baseline before BMS-663068 therapy. No known resistance mutations were selected for in any of the other subjects.

Examination of the relationship between changes in FC-IC_{50} and baseline parameters revealed that greater increases in FC-IC_{50} on day 8 may be more likely in those subjects with high baseline FC-IC_{50} (Fig. 3A). There was no apparent relationship between baseline plasma HIV-1 RNA level or maximum reduction in plasma HIV-1 RNA and change from baseline of FC-IC_{50} on day 8 (Figs. 3B, C, respectively).

DISCUSSION

BMS-626529, dosed as the prodrug BMS-663068, has demonstrated antiviral activity in a short-term monotherapy study in HIV-1–infected subjects.[7] The data presented here demonstrate that virological nonresponse to BMS-663068 was predicted by low baseline susceptibility to BMS-626529 (reflected by a high baseline FC-IC_{50}).

The prominence of gp120, an extracellular protein, as a major target of immune responses in the host, likely results in considerable immune-based selective pressures on gp120. This in turn results in considerable changes in gp120 sequence and greater heterogeneity within the infecting quasispecies with longer duration of infection, greater virological turnover, or more progressive disease. Changes in HIV-1 tropism

(mediated by changes in gp120) from CCR5 to CXCR4 or dual-tropic virus have been noted with longer duration of infection, higher plasma HIV-1 RNA levels, and lower $CD4^+$ T-cell count.[15] Similar changes in the gp120 population may in turn be relevant to the in vivo antiviral activity of BMS-626529. We explored whether variables related to these factors influenced antiviral activity in our study. We did not observe any obvious correlation between virological response to BMS-626529 and history of previous antiretroviral experience, baseline plasma HIV-1 RNA level, or baseline $CD4^+$ T-cell count. Similarly, we found no correlation with coreceptor tropism in this clinical study, in line with our previous in vitro findings of the lack of relationship between coreceptor usage and BMS-626529 susceptibility.[6]

In an earlier in vitro study, several substitutions were found to be associated with decreased susceptibility to BMS-626529.[11] After manual sequence examination at these positions in subjects enrolled in this study, M426L was found to be the primary substitution whose presence at baseline was associated with nonresponse to BMS-626529. The M426L substitution was absent at baseline in 1 nonresponder, who instead harbored substitutions at other positions associated with resistance to BMS-626529 and BMS-448043 (M434I and S375M). M426L was also found to be present in 2 responders. Therefore, we conclude that while the presence of the M426L substitution at baseline does not entirely preclude response, it does seem to be predictive of nonresponse to BMS-663068.

In this study, an 8-day monotherapy did not select for high-level resistance to BMS-626529. Furthermore, the median FC-IC_{50} between days 1 and 8 was minimal and similar across treatment groups, and there was no apparent relationship between baseline plasma HIV-1 RNA levels or virological response and fold change of FC-IC_{50} on day 8. Possible increases in the proportion of L426 were observed in the envelope populations for 2 subjects and may represent the effect of drug pressure by BMS-626529, resulting in enrichment of envelopes with L426 or suppression of M426.

The lack of emergence of high-level resistance observed in the current study is in contrast to an earlier study of BMS-488043, in which viruses from 4 of 24 subjects who received BMS-488043 had emergent resistance [>10-fold change in susceptibility from baseline (range: 33- to 344-fold)] by day 8.[4] This difference may be due to one or more factors differentiating BMS-626529 from BMS-488043. BMS-626529 has demonstrated greater in vitro antiviral potency compared with BMS-488043 for the majority of HIV-1 strains tested.[6] Also, blood levels of BMS-626529, administered as the prodrug BMS-663068, were higher than those of BMS-488043.[5,7] The increased in vitro antiviral potency combined with the higher blood levels of BMS-626529 are likely to produce greater inhibitory quotients, which may limit the likelihood and speed of resistance selection, and result in an increased barrier to resistance for BMS-626529 compared with BMS-488043.

Emergence of resistance has been reported in short-term monotherapy studies of other entry inhibitors, such as maraviroc and enfuvirtide. In a 10-day monotherapy study of maraviroc, changes in virus tropism were observed in 2 of 8 subjects receiving a 100 mg once daily dose of maraviroc, although viral load responses in these individuals were comparable to those of other subjects in the treatment group.[16] In a 14-day enfuvirtide monotherapy study, viral resistance emerged in 2 of 4 subjects in the 30 mg (subtherapeutic dose) arm but not in any of the other treatment arms.[17] Longer-term (28-day) monotherapy studies of enfuvirtide, showed high-level (>10-fold) increases in IC_{50} in >20% of evaluable subjects at the end of the study period.[18,19]

Our study has certain limitations. The small number of subjects limits the robustness of some of the conclusions. For example, the lack of correlation of virological response with some baseline variable (such as plasma HIV-1 RNA or $CD4^+$ T-cell count) noted in this exploratory study will need to be confirmed in larger clinical trials with more heterogeneous subject populations. Furthermore, larger populations will be required to better define a clinically meaningful phenotypic resistance cutoff or a genotypic algorithm that can predict virological response. The highly polymorphic nature of gp120 observed across clinical isolates may suggest a considerably greater heterogeneity in gp120 within the quasispecies prevalent in an infected subject compared with other viral targets. Our resistance analysis here focused on population phenotyping or genotyping due in part to the ready availability of these methods for clinical assessment now and in the near future. Nevertheless, a comprehensive evaluation of minority variants at baseline to understand their role in virological response will be informative. Assessment of changes in minority variants at the end of therapy will also be helpful to understand the viral quasispecies' response to selective pressure with BMS-626529. The apparent increases in the proportion of L426 observed in the envelope populations for 2 subjects who experienced limited increases in the FC-IC_{50} is consistent with BMS-626529–driven enrichment of minority L426.

In conclusion, nonresponse to BMS-663068 was associated with low baseline susceptibility to BMS-626529 and the presence of AI resistance substitutions such as M426L, S375M, and M434I. In this short-term monotherapy trial, there was minimal evidence of selection for high-level BMS-626529 resistance as measured by standard population-based phenotypic and genotypic approaches. These data support the continued clinical development of BMS-626529, and a phase 2b study of BMS-663068 in HIV-1–infected treatment-experienced subjects is ongoing (NCT01384734).

ACKNOWLEDGMENTS

The authors would like to acknowledge Richard Nettles, Shu-Pang Huang, Peter Hu, and Joachim Dejonckheere for their contributions to the study design and data analysis. Editorial assistance was provided by Clemence Hindley of MediTech Media and was funded by Bristol-Myers Squibb. Trial registration: ClinicalTrials.gov identifier: NCT01009814.

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