The Second-Generation Maturation Inhibitor GSK3532795 Maintains Potent Activity Toward HIV Protease Inhibitor–Resistant Clinical Isolates

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Background: Protease inhibitor (PI)-resistant HIV-1 isolates with primary substitutions in protease (PR) and secondary substitutions in Gag could potentially exhibit cross-resistance to maturation inhibitors. We evaluated the second-generation maturation inhibitor, GSK3532795, for activity toward clinical isolates with genotypic and phenotypic characteristics associated with PI resistance (longitudinal).

Methods: Longitudinal clinical isolates from 15 PI-treated patients and 7 highly PI-resistant (nonlongitudinal) viruses containing major and minor PI resistance-associated mutations were evaluated for GSK3532795 sensitivity. Phenotypic sensitivity was determined using the PhenoSense Gag/PR assay (Monogram Biosciences) or in-house single- and multiple-cycle assays. Changes from baseline [CFB; ratio of post- to pre-treatment FC-IC50 (fold-change in IC50 versus wild-type virus)] <3 were considered to be within the no-effect level.

Results: All nonlongitudinal viruses tested were sensitive to GSK3532795 (FC-IC50 range 0.16–0.68). Among longitudinal isolates, all post-PI treatment samples had major PI resistance-associated mutations in PR and 17/21 had PI resistance-associated changes in Gag. Nineteen of the 21 post-PI treatment samples had GSK3532795 CFB <3. Median (range) CFB was 0.83 (0.05–27.4) [Monogram (11 patients)] and 1.5 (1.0–2.2) [single-cycle (4 patients)]. The 2 post-PI treatment samples showing GSK3532795 CFB >3 were retested using single- and multiple-cycle assays. Neither sample had meaningful sensitivity changes in the multiple-cycle assay. Gag changes were not associated with an increased GSK3532795 CFB.

Conclusions: GSK3532795 maintained antiviral activity against PI-resistant isolates with emergent PR and/or Gag mutations. This finding supports continued development of GSK3532795 in treatment-experienced patients with or without previous PI therapy.

Key Words: HIV-1, maturation inhibitor, GSK3532795, protease inhibitor, cross-resistance, in vitro

INTRODUCTION

HIV and AIDS remain a global health issue despite the success of combination antiretroviral (ARV) therapy.1 Life-long management of HIV-1 infection requires sequential ARV therapies, preferably with simple and convenient regimens containing at least 2 fully active agents.2,3 ARV treatment options, particularly for treatment-experienced patients, may be limited by treatment-emergent or transmitted resistance, adverse events, drug–drug interactions, or regimen complexity.2–4 Therefore, novel ARVs are needed that could potentially change HIV-1 treatment paradigms. Such regimens would benefit from components with novel mechanisms of action, unique resistance profiles, good long-term tolerability, and manageable drug–drug interactions.

HIV-1 maturation is the final step in the viral life cycle and involves multiple cleavages by the viral protease (PR) at discrete sites in HIV-1 Gag, leading to a profound morphologic rearrangement of the virion and condensation of the viral capsid (CA) core with concomitant release of infectious virus from the host cell.5,6 Disrupting Gag cleavage at individual sites results in the production of noninfectious HIV-1 particles,7,8 suggesting that inhibition of HIV-1 maturation might represent a novel therapeutic approach. Bevirimat (BVM) was a first-generation maturation inhibitor (MI) that inhibited the last proteolytic cleavage event in Gag, between the p24 CA protein and spacer peptide 1 (SP1), thereby resulting in the production of immature, noninfectious virus particles.9–12 Phase 2 studies of
BVM provided proof of concept for this class of agents by demonstrating dose-dependent antiviral activity.7 However, phase 2 development revealed that approximately 50% of patients did not respond to treatment, which was associated with naturally occurring polymorphisms in HIV-1 Gag13 at or near its site of activity.14 GSK3532795 (formerly BMS-955176) is a second-generation MI that also inhibits this single, specific HIV-1 PR cleavage event between CA and SP1 in Gag, producing immature, noninfectious virus particles. However, it exhibits potent activity toward the polymorphic variations in Gag associated with resistance to BVM.15,16

Protease inhibitors (PIs), a widely used class of ARVs, block HIV-1 replication by binding to viral PR, thereby preventing all Gag cleavage events. Clinically, PIs select for PI-resistance mutations that map to viral PR but they can also select for PI-resistance mutations that map to the p7/SP2 and SP2/p6 regions of Gag (amino acids 431–453; hereafter termed “Gag PR-resistance mutations”).17–22 Although sites for Gag PI-resistance mutations are distinct from those reported for BVM,23–26 there have been conflicting reports about the prevalence of BVM resistance-associated Gag polymorphisms in patients on PI therapy.17,24,27,28 Although GSK3532795 has demonstrated potent in vitro activity toward HIV-1 strains containing BVM resistance-associated Gag polymorphisms,16 there is still a need to rigorously determine the sensitivity of PI-resistant viruses to GSK3532795. This will also be clinically relevant for the use of GSK3532795 in patients with PI-treatment experience or failure.

In this first detailed study examining possible cross-resistance between MI and PI ARVs, we investigated whether the acquisition of PI resistance altered GSK3532795 sensitivity. Two sets of highly PI-resistant HIV-1 clinical isolates were investigated. PR only (nonlongitudinal) isolates or PR and Gag (longitudinal isolates, PR and Gag genes derived from patients who were PI-naive at baseline but acquired PI resistance while on PI therapy) were transferred into a laboratory backbone virus for antiviral testing. Here, we report that GSK3532795 maintains antiviral activity against PI-resistant isolates with emergent PR that GSK3532795 maintains antiviral activity against PI-resistant isolates with emergent PR and/or Gag mutations, thus supporting its continued development in treatment-experienced patients regardless of previous PI therapy.

**METHODS**

**Compounds**

GSK3532795, BMS MI A, BMS MI B, and atazanavir (ATV) were prepared at Bristol-Myers Squibb. BMS MI A and B were evaluated as they are structurally related to GSK3532795, allowing for a determination of the generality of the overall response of PI-resistant isolates to MIs beyond GSK3532795. Darunavir (DRV) and lopinavir (LPV) were purchased and purified from commercial sources.

**Cells and Viruses**

MT-2 and HEK 293T cells, and the proviral DNA clone of NL4-3 were obtained from the NIH AIDS Research and Reference Reagent Program. Cell lines were subcultured twice a week in either RPMI 1640 (MT-2) or DMEM (HEK 293T) media supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin G, and 100 μg/mL streptomycin.15

The proviral plasmid pNLRepRluc was constructed at Bristol-Myers Squibb from the proviral NL4-3 clone and contained Renilla luciferase in place of the viral nef gene.15

The Gag/PR regions from longitudinal clinical isolates were originally amplified by reverse transcriptase–polymerase chain reaction (PCR). All amplicons were sequenced and, in cases in which cloning and/or assay failures at Monogram Biosciences precluded phenotypic analysis, longitudinal amplicons were reamplified and the Gag/PR regions cloned into pNLRepRluc for evaluation in the Bristol-Myers Squibb multiple- or single-cycle assays (described in section “Susceptibility Assays Performed at Bristol-Myers Squibb”).

A set of nonlongitudinal clinical isolates containing multiple PI-resistance mutations29 was analyzed for sensitivity to GSK3532795, BMS MI B, LPV, and ATV in the multiple-cycle assay. Isolates [NL4-3 background, PI-resistance mutations in PR, wild-type NL4-3 Gag] were obtained from Dr. Robert Shafer (Stanford University) through the NIH AIDS Research and Reference Reagent program, and the Gag/PR regions cloned into pNLRepRluc.

**Drug Susceptibility Assays**

**Susceptibility Assays Performed at Monogram Biosciences**

Monogram Biosciences performed susceptibility assays using the PhenoSense HIV-1 Gag/PR assay (hereafter called the Monogram assay), which is a pseudotype-based, single-cycle assay.30 DRV was chosen as the representative PI to test susceptibility of the isolates. Recombinant virus stocks [pseudotyped with amphotropic murine leukemia virus env proteins] were produced by cotransfecting HEK 293T cell cultures with amphotropic murine leukemia virus env and pHIVlc-resistance test vectors. Viral stocks were deposited in 96-well plates containing serial dilutions of PIs spanning an empirically determined range for each drug. Viral stocks were harvested approximately 48 hours after transfection and used to inoculate fresh HEK 293T cell cultures. Replication was monitored by measuring luciferase expression in the infected target HEK 293T cells ~72 hours after infection.

Drug susceptibility data were determined by plotting the percent inhibition of luciferase activity versus log_{10} drug concentration.15 The fold-change in drug susceptibility (FC-I_{C50}) was determined by dividing the drug concentration leading to 50% viral inhibition (I_{C50}) values for the Gag/PR recombinant virus by those of a drug-sensitive reference virus containing the Gag/PR sequences of NL4-3.

**Susceptibility Assays Performed at Bristol-Myers Squibb**

Single-cycle pseudotype-based (hereafter called the single-cycle assay) and multiple-cycle susceptibility assays were performed at Bristol-Myers Squibb. ATV and LPV were chosen as the representative PIs to test susceptibility of the isolates. For the single-cycle assay, 10 μg of full-length
## TABLE 1. Treatment History, Genotype, and Predicted Phenotypic Susceptibility of all Longitudinal Samples

| Sample Name | PIs in ARV Regimen | Years on PI Therapy | PR Genotype (Primary PI Mutations in PR) | Predicted Resistance to PI (Based on HIV Drug Resistance Database) |
|-------------|---------------------|---------------------|------------------------------------------|---------------------------------------------------------------|
|             |                     |                     | Major | Minor | A17V | DRV | E6V | E69 | L90 | L90I | G73S |
| pD2 pre-PI  |                     |                     | None  | L10I  |      |     |     |     |      |      |      |
| pD2 PTx 1   |                     |                     | 6.8   |       | M46I | L90I | G73C |
| pD2 PTx 2   |                     |                     | 11.2  |       | M46I | L90I | G73C |
| pD3 pre-PI  |                     |                     | None  | A17V  |      |     |     |     |      |      |      |
| pD3 PTx 1   |                     |                     | 3.1   |       | L93M | A17V | G73S |
| pD4 pre-PI  |                     |                     | None  | None  |      |     |     |     |      |      |      |
| pD4 PTx 1   |                     |                     | 5.7   |       | M46I | L90I | G73C |
| pD4 PTx 2   |                     |                     | 10.7  |       | M46I | L90I | L24I |
| pD5 pre-PI  |                     |                     | None  | L10I  | A17V |     |     |     |      |      |      |
| pD6 PTx 1   |                     |                     | 10.6  |       | I54V | L90I | L24I |
| pD6 PTx 2   |                     |                     | 4.2   |       | V10I | L90I | L24I |
| pD7 pre-PI  |                     |                     | None  | None  |      |     |     |     |      |      |      |
| pD8 PTx 1   |                     |                     | 0.8   |       | V10I | L90I | A17V |
| pD8 PTx 2   |                     |                     | 4.2   |       | V10I | L90I | A17V |
| pD9 pre-PI  |                     |                     | None  | C073 | A17V |     |     |     |      |      |      |
| pD10 PTx 1  |                     |                     | 5.9   |       | D30N | L90I | A17V |
| pD11 pre-PI |                     |                     | None  | L10I  | E35D |     |     |     |      |      |      |
| pD11 PTx 1  |                     |                     | 3.8   |       | M46I | E35D | N88D |
| pD11 PTx 2  |                     |                     | 10.7  |       | M46I | L90I | L24I |
| pD12 pre-PI |                     |                     | None  | L10I  |      |     |     |     |      |      |      |
| pD13 PTx 1  |                     |                     | 2.7   |       | N85S | L90I | A17V |
| pD14 PTx 1  |                     |                     | None  | None  |     |     |     |     |      |      |      |
| pD15 PTx 1  |                     |                     | 2.3   |       | M46I | V10I | A17V | G73S |
| pD16 PTx 2  |                     |                     | None  | None  |     |     |     |     |      |      |      |
| pD17 PTx 1  |                     |                     | 6.5   |       | M46I | V10I | L90I |
| pD18 PTx 1  |                     |                     | 11.7  |       | M46I | L90I | V111T |

**Note:** Resistance levels: susceptible; high; low; intermediate; and other.

ARV, antiretroviral; ATV, atazanavir; DRV, darunavir; FPV, fosamprenavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; PI, protease inhibitor; PI<sup>r</sup>, protease inhibitor resistance; PR, protease; PTx, post-PI treatment; pt, patient; lr, low-dose ritonavir; SQV, saquinavir; TPV, tipranavir.

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pNLRepRuc variant (containing Gag/PR genes from clinical isolates) and 8 μg of plasmid SV-A-MuLV-env were cotransfected into HEK 293T cells in T75 flasks using a calcium precipitation method (Thermo Fisher Scientific). Transfected cells (100 μL) were seeded onto 96-well plates which contained 100 μL of compound dilutions and after ~30 hours, 100 μL of supernatant (containing newly produced virus) was transferred to freshly cultured HEK 293T cells and maintained for 2 days. Cell-associated Renilla luciferase activity was measured by the addition of EnduRen Live Cell Substrate (Promega). For multiple-cycle assays, MT-2 cells were infected with pooled full-length virus-containing Gag/PR genes from clinical isolates in the pNLRepRuc backbone versus wild-type control virus. Cell–virus mixtures were seeded onto 96-well plates containing serially diluted compounds. After 4 days’ incubation at 37°C/5% CO2, virus growth was determined by measuring the activity of cell-associated Renilla luciferase as described above.

Longitudinal isolates (pre- and post-PI treatment) were obtained from 15 patients receiving PIs as part of their combination ARV therapy regimen for a median (range) of 6 (2.3–11.7) years (Table 1). There were 21 post-PI treatment samples collected while patients were on PI therapy (9 patients had 1 post-treatment sample and 6 patients had 2 post-PI treatment samples). Cloning of Gag/PR amplicons from all 15 patients was performed by Monogram Biosciences. However, only samples from Pts02, 03, 04, 06, 07, 09, 10, 11, 12, 14, and 15 produced pooled clones that could be analyzed for phenotypic sensitivity to GSK3532795 and a clinically relevant PI (DRV). Some pre- or post-PI treatment samples, or both, from Pts01, 05, 06, 08, and 16 yielded a nonreportable result from the Monogram assay but were recloned at Bristol-Myers Squibb and analyzed for phenotypic susceptibility to GSK3532795, BMS MI A and BMS MI B, and 2 clinically relevant PIs (ATV and LPV). For samples in which a potentially elevated GSK3532795 susceptibility was identified by the Monogram assay [changes from baseline (CFB) >3-fold], additional cloning and re-analysis was performed using the single- and multiple-cycle assays (Supplemental Digital Content, Fig. 1 and Table 2 http://links.lww.com/QAI/A974).

Assay Interpretation

All phenotypic data from longitudinal isolates were expressed as CFB and calculated as the ratios of post- to pre-PI treatment FC-IC50 values (data not shown). Based on similar phenotyping assays (eg, Monogram Biosciences PhenoSense Entry assay), CFB values >3 were considered within the “no-effect” range, and CFB values >3 were

| TABLE 2. Longitudinal Isolates (Monogram Assay): GSK3532795 and DRV Phenotypic Susceptibility, and Gag Genotype |
| Sample Name | GSK3532795 FC-IC50 | DRV | GSK3532795 FC-IC50 | DRV | GSK3532795 CFB | DRV | GSK3532795 CFB | DRV |
|-------------|-------------------|-----|-------------------|-----|----------------|-----|----------------|-----|
| Pt02 pre-PI | 2.13              | 2.84| Pt02 PTx 1        | 1.27| 1.59           | Pt02 PTx 2        | 1.06| 0.93           |
| Pt03 pre-PI | 0.62              | 1.40| Pt03 PTx 1        | 0.63| 0.98           | Pt03 PTx 1        | 0.39| 1.37           |
| Pt04 pre-PI | 1.47              | 1.48| Pt04 PTx 1        | 0.48| 1.88           | Pt04 PTx 1        | 0.70| 2.78           |
| Pt06 pre-PI | 0.89              | 1.15| Pt06 PTx 2        | 7.46| 3.50           | Pt06 PTx 2        | 10.97| 5.18          |
| Pt07 pre-PI | 0.76              | 0.75| Pt07 PTx 1        | 0.39| 2.89           | Pt07 PTx 1        | 0.30| 2.17           |
| Pt09 pre-PI | 0.43              | 1.19| Pt09 PTx 2        | 27.45| 2.93         | Pt09 PTx 2        | 1.37| 13.00          |
| Pt10 pre-PI | 0.54              | 1.45| Pt10 PTx 1        | 1.37| 1.45           | Pt10 PTx 1        | 0.55| 4.28           |
| Pt11 pre-PI | 22.30             | 1.85| Pt11 PTx 2        | 0.56| 0.58           | Pt11 PTx 2        | 2.26| 2.63           |
| Pt12 pre-PI | 0.24              | 0.67| Pt12 PTx 1        | 0.39| 0.39           | Pt12 PTx 1        | 0.30| 2.17           |
| Pt13 pre-PI | 58.36             | 2.00| Pt13 PTx 2        | 0.56| 0.58           | Pt13 PTx 2        | 1.12| 1.07           |
| Pt14 pre-PI | 58.36             | 2.00| Pt14 PTx 1        | 0.23| 1.96           | Pt14 PTx 1        | 256.82| 1.02         |
| Pt15 pre-PI | 1.09              | 0.79| Pt15 PTx 2        | 0.48| 5.24           | Pt15 PTx 2        | 0.52| 4.14           |

CFB, change from baseline; DRV, darunavir; FC, fold-change; MI, maturation inhibitor; PI, protease inhibitor resistance; PTx, post-PI treatment; Pt, patient.
considered to be indicative of an effect on susceptibility. The relevance of this CFB cut-off in predicting GSK3532795 clinical efficacy is yet to be determined.

RESULTS

Phenotypic Susceptibilities of Highly Resistant PR Genes to GSK3532795: Nonlongitudinal Isolates

Consistent with their genotypic profiles, NLRepRluc proviruses expressing PR genes from a panel of 7 publicly available HIV-1 viruses containing multiple major and minor primary PI resistance-associated mutations (RAMs) were resistant to LPV (FC-IC50 range 15–442) and ATV (FC-IC50 range 11–415), but retained susceptibility to GSK3532795 and BMS MI B, with FC-IC50 values <1 (Supplemental Digital Content, Table 1 http://links.lww.com/QAI/A974). A virus with the A364V Gag substitution was used as a positive control for reduced GSK3532795 susceptibility. These data clearly indicate that highly PI-resistant viruses with PI RAMs in PR retain sensitivity to GSK3532795 and to a second structurally-related BMS MI (MI B).

Genotypic and Phenotypic Characteristics of Highly Resistant PR and Gag Genes: Longitudinal Isolates

Gag and PR genes from longitudinal isolates from 15 PI-resistant patients were cloned into recombinant virus vectors. Baseline (pre-PI treatment) samples, except those from patient 10 (Pt10), which contained a D30N mutation, contained no major PI RAMs. Conversely, all post-PI treatment samples had major PI RAMs in PR (Table 1) and 16/27 samples had PI-resistant mutations in Gag (at amino acid positions 128, 431, 436, 437, 449, 452, and 453) (Table 2, see also Supplemental Digital Content, Fig. 2, http://links.lww.com/QAI/A974 for full sequences of the entire Gag/PR region). In several patient samples (Pts06, 07, 09, and 15; Tables 2 and 3), changes were acquired in Gag at or near the purported site of action of MIs (near the CA/SP1 cleavage site). Ten of the 27 pre- or post-PI treatment samples had polymorphisms at Gag amino acids 362, 369, or 370, which are associated with BVM resistance. The phenotypic susceptibilities of these samples to 8 commonly used PIs [ATV, DRV, LPV, saquinavir (SQV), tipranavir (TPV), fosamprenavir (FPV), indinavir (IDV), and nelfinavir (NFV)] were predicted based on their PR genotype using the Stanford HIV Database Genotypic Resistance Interpretation Algorithm (Table 1).

Pre-PI treatment samples from 14/15 patients were predicted to be either completely susceptible or exhibit only low resistance to all 8 PIs. Based on its D30N mutation, the sample from Pt10 was predicted to be susceptible to 7 PIs but highly resistant to NFV. All post-PI treatment samples were predicted to have intermediate/high resistance to ≥3 PIs.

Phenotypic Susceptibilities of All Longitudinal Isolates to GSK3532795

All pre- and post-PI treatment samples from 15 patients were analyzed by Monogram Biosciences for phenotypic susceptibility to GSK3532795 and DRV. The Monogram assay successfully reported results for at least 1 post-PI therapy time point from 11/15 patients (Pts02, 03, 04, 06, 07, 09, 10, 11, 12, 14, and 15). For Pt06, results were reported from the pre-PI treatment and only the second of 2 post-PI treatment samples. As shown in Figure 1, major PI RAMs were associated with a 1.9–4.37-fold increase in the median DRV CFB in post-PI treatment samples. The FC-IC50 median (range) was 1.19 (0.67–2.84) for pre-PI treatment samples and 2.72 (0.39–13.00) for post-PI treatment (FC-IC50 data not shown in Fig. 1). Although none of the samples had FC-IC50 >90, indicating clinically defined DRV resistance, the second post-PI treatment sample from Pt09 showed intermediate DRV resistance (FC-IC50 = 13). GSK3532795 susceptibility was observed in
9/11 pre-PI treatment samples, whereas low susceptibility to 
GSK3532795 was observed in the other 2 patients (FC-IC50 
Pt11: 22.30; Pt14: 256.82). The determinants for reduced 
GSK3532795 susceptibility are currently being further exam-
ined in these samples and are not yet understood. However, the 
corresponding post-PI treatment samples had greatly reduced 
FC-IC50, indicating enhanced susceptibility. Overall, although 
no consistent change was observed in the distribution of the 
GSK3532795 FC-IC50s within this set, the distribution of the 
GSK3532795 FC-IC50s in post-PI treatment samples indicates 
that the presence of major PI RAMs did not reduce 
GSK3532795 susceptibility (Table 2 and Fig. 1).

GSK3532795 and DRV susceptibilities of patient samples 
with reportable results from the Monogram assay were calculated 
as CFB (Table 2 and Fig. 1). Median CFB for DRV was 
generally >3 and increased in the 6 patients with multiple post-
PI treatment samples from the first (median CFB = 1.92) to the 
second (median CFB = 4.37) sample, suggesting decreasing drug 
susceptibility with greater PI treatment experience (Fig. 1 and 
Table 2). These observations are consistent with the genotype 
data (Table 1), which show that inpatients with multiple post-PI 
treatment samples, predicted resistance to one or more PIs 
increases from the 
first to the second sample. Median CFB for 
GSK3532795 was 
1; suggesting minimal change for both the 
first (median CFB = 0.6) and second (median CFB = 1.33) sets 
of post-PI treatment samples. However, one of each of 2 time 
point samples from Pt04 and Pt09 had GSK3532795 CFB 
(further analysis of these samples is presented in section 
"Further Analysis of Longitudinal Isolates With GSK3532795 CFB ").
Conversely, samples from Pts11, 14, and 15 seemed to show 
increased susceptibility to GSK3532795 compared with pre-PI 
treatment (CFB , 0.33) (Table 2). The genotype data indicate

### TABLE 3. Longitudinal Isolates (Single- and Multiple-Cycle Assays): GSK3532795 and PI Phenotypic Susceptibility, and Gag 
Genotype* 

| Sample Name      | CF-IC50 | FC-IC50 |
|------------------|---------|---------|
|                  | GSK3532795 | ATV | BMS MI A | BMS MI B | LPV | BMS MI A | BMS MI B | LPV |
| Pt01 pre-PI      | 1       | 1      | 1      | 1      | 1   | 0.90    | 0.33    | 0.41 |
| Pt01 PTx         | 2       | 0.42   | 0.51   | 7.4    | 3   | 1.8     | 0.14    | 0.21 |
| Pt05 pre-PI      | 1       | 1      | 1      | 1      | 1   | 2.9     | 2.8     | 1.4  |
| Pt05 PTx         | 0.4     | 0.2    | 0.5    | 5.3    | 4.6 | 1.0     | 0.6     | 0.7  |
| Pt06 pre-PI      | 1       | 1      | 1      | 1      | 1   | 1.1     | 0.44    | 0.71 |
| Pt06 PTx 1       | 2.5     | 2.2    | 1.4    | 4.2    | 75.9| 2.2     | 1.0     | 1.0  |
| Pt06 PTx 2       | 1.4     | 0.92   | 0.87   | 3.4    | 18.5| 1.5     | 0.41    | 0.61 |
| Pt08 pre-PI      | 1       | 1      | 1      | 1      | 1   | 2.6     | 0.94    | 0.75 |
| Pt08 PTx         | 0.49    | 0.2    | 0.36   | 2.9    | 1.1 | 1.3     | 0.19    | 0.27 |
| Pt10 pre-PI      | 1       | 1      | 1      | 1      | 1   | 0.89    | 0.45    | 0.66 |
| Pt10 PTx         | 0.83    | 0.85   | 0.60   | 4.5    | 4.0 | 0.68    | 0.39    | 0.40 |
| Pt16 pre-PI      | 1       | 1      | 1      | 1      | 1   | 1.3     | 0.33    | 0.49 |
| Pt16 PTx         | 1.7     | 1.1    | 1.8    | 28.9   | 31.2| 1.8     | 0.35    | 1.0  |

*Samples from pt10 were recloned and retested as a control for the single-cycle assay. All post-treatment samples contain ≥1 major PI RAM.

ATV, atazanavir; CFB, change from baseline; LPV, lopinavir; MI, maturation inhibitor; PP*, protease inhibitor resistance; PTx, post-PI treatment; pt, patient; RAM, resistance-
associated mutation; SP1, spacer peptide-1.
that these samples had intermediate or high predicted resistance to all PIs except DRV (Table 1).

Samples from patients with nonreportable results (Pts01, 05, 06, 08, and 16) using the Monogram assay were recloned and analyzed using the single-cycle assay for phenotypic susceptibilities to GSK3532795, BMS MI A and BMS MI B, and the clinically relevant PIs LPV and ATV. Pt10 samples were used as a control for cross-comparison purposes. The presence of major PI RAMs in 5 post-PI treatment samples from 4 of these patients was associated with ATV and/or LPV CFB values >3, indicative of PI resistance (Table 3, Supplementary Digital Content, Fig. 2, http://links.lww.com/QAI/A974). The exception was Pt08, whose major RAM was D30N (characteristic of NFV resistance) and thus still showed susceptibility to ATV and LPV. All post-PI treatment samples had CFB <3 for GSK3532795, BMS MI A, and MI B CFB (Table 3, Supplementary Digital Content, Fig. 2, http://links.lww.com/QAI/A974).

In summary, analysis of these longitudinal samples demonstrates a lack of cross-resistance to GSK3532795 in the presence of high-level PI resistance and PI treatment-induced mutations in Gag. The observation of sensitivity to MIs A and B further generalizes this result of lack of cross-resistance of MIs to PR-resistant isolates (Table 3, Supplemental Digital Content, Fig. 2, http://links.lww.com/QAI/A974).

Further Analysis of Longitudinal Isolates With GSK3532795 CFB >3

The first and second post-PI treatment samples from Pts09 and 04, respectively, had GSK3532795 CFB values substantially >3, and were subsequently recloned and tested using both single- and multiple-cycle assays. The first post-PI treatment sample from Pt09 showed GSK3532795 CFB ~1.5 in both assays. The second post-PI treatment sample from Pt04 reproduced a CFB >3 (4.17, n = 2 independent experiments) in the single-cycle assay and CFB <3 (2.1) in the multiple-cycle assay. Both were resistant to ATV and/or LPV in the single-cycle assay (Pt04 time point 2 FC-IC50: ATV = 402; LPV = 152; Pt09 time point 1 FC-IC50: ATV = 14.8; LPV = 9.6) and the multiple-cycle assay (Pt04 time point 2 FC-IC50: ATV = 217, LPV = 133; Pt09 time point 1 FC-IC50: ATV = 4.4, LPV = 5.1) (Supplemental Digital Content, Table 2, http://links.lww.com/QAI/A974). Thus, the data suggest that the post-PI treatment samples from Pt04 and Pt09 did not exhibit a significant CFB toward GSK3532795.

Impact of PI-Resistance Mutations in Gag Cleavage Sites on GSK3532795 Susceptibility

The most frequently observed mutations in the Gag polyprotein shown to affect PI susceptibility are in MA/CA (codons 128, 431, 436, and 437), and SP2/P6 (codons 449, 452, and 453).37-22 None of these mutations map to amino acids associated with BVM susceptibility.3 Among the samples analyzed using the Monogram assay, ≥1 Gag PI-resistance mutation (except for a change in codon 452) was present in ≥1 post-PI treatment sample from 10/11 patients. Although samples with Gag PI-resistance mutations had a wider range of GSK3532795 CFB values, median values were similar regardless of the presence of these mutations. In contrast, median DRV CFB values were higher when Gag PI-resistance mutations were present (median CFB = 2.94) than not (median CFB = 1.26) (Fig. 2). Samples analyzed using the single- or multiple-cycle assays had ≥2 Gag PI-resistance mutations in 4/5 patients (excluding P08) (Table 1). GSK3532795, BMS MI A, and BMS MI B CFB values for these samples were similar regardless of the presence of these mutations.

Changes in Gag at or near the site of MI action, near CA/SP1, were observed in Pts06, 07, 09, and 15 (Tables 2 and 3). Despite the presence of these Gag changes, some of which have been associated with BVM resistance, and could thus be associated with resistance to other MIs, post-PI treatment samples from these patients remained susceptible to GSK3532795 and other structurally related-MIs.

DISCUSSION

MIs inhibit the final PR-mediated cleavage event in Gag, between the CA protein and SP1, whereas PIs inhibit all the
PR-mediated cleavage steps required for virus maturation. Given the related mechanisms of action of these agents, there is potential for emergent PI RAMs to reduce MI susceptibility. This is the first comprehensive study to examine in detail the potential for cross-resistance between MI and PI ARVs. Using both nonlongitudinal (containing only the PR genes and mutations within) and longitudinal (containing PR and Gag genes and mutations within) clinical isolates from patients with acquired PI resistance, we found no definitive examples of viruses exhibiting reduced susceptibility to GSK3532795 in the presence of baseline or progressive PI resistance. Larger sample sizes will be helpful to support these findings.

Analysis of 7 nonlongitudinal HIV-1 viruses containing highly PI-resistant PR genes with multiple major and minor PI RAMs showed that these mutations were not associated with reduced sensitivity to GSK3532795. In a converse analysis, PI susceptibility was studied in viral isolates that exhibited reduced GSK3532795 susceptibility. As expected, viruses with reduced GSK3532795 susceptibility (FC-IC50 3.3–67) retained susceptibility to DRV, LPV, ATV, and NFV (unpublished data, Bristol-Myers Squibb). These data suggest that previous use of PIs will not affect subsequent use of GSK3532795 in PI—treatment-experienced patients and vice versa.

Pre- and post-PI treatment samples of all longitudinal clinical isolates were genotyped and predictions performed on their susceptibility to 8 PIs. As the patients were PI-naive at baseline, major PI RAMs were not present in most of the pre-PI treatment samples, but were present in all the post-PI treatment samples and associated with a predicted reduction in susceptibility to DRV, ATV, LPV, and a number of other less commonly used PIs. Phenotypic susceptibilities to PIs (DRV, ATV, and LPV) and MI (GSK3532795, BMS MI A, and BMS MI B) were determined using a combination of Monogram and BMS single- and multiple-cycle susceptibility assays. The Monogram assay reported that longitudinal clinical isolates from 9/11 patients, except the first and second post-PI treatment samples for Pts09 and 04, respectively, retained susceptibility to GSK3532795 even in the presence of major PI RAMs. Pre- and/or post-PI treatment samples from Pts01, 05, 06, 08, and 16 yielded a nonreportable result from the Monogram assay and were thus re-analyzed (BMS single- and multiple-cycle assays). As for the set analyzed by Monogram, despite the high-level PI resistance mediated by PR and Gag RAMs, these samples remained susceptible to GSK3532795.

As the predicted PI-resistance profiles of samples from Pts04 and 09 were similar to others within the same set, further phenotypic analyses were performed to verify the Monogram GSK3532795 results. However, the single- and multiple-cycle assays showed that the first post-PI treatment sample for Pts09 had no significant change in susceptibility (CFB <3) to GSK3532795 and BMS MI A and BMS MI B. The second post-PI treatment sample for Pts04 had variable results: GSK3532795 CFB was >3 using the single-cycle assay but <3 using the multiple-cycle assay. The Monogram and BMS single- and multiple-cycle assays used the same primary PCR product for cloning and, additionally, positive control samples from Pts10 produced the same results from both the Monogram and BMS single-cycle assays. Thus, we speculate that differences in results between the assays might be attributable to small differences in PCR reamplification before cloning or small differences in assay conditions, although this was not formally tested. In summary, this detailed analysis generally showed that samples from Pts04 and Pts09 remained susceptible to GSK3532795.

The impact of PI-resistance mutations near the C-terminus of Gag on GSK3532795 susceptibility was also tested. Median GSK3532795 CFB values were similar in the presence or absence of such Gag PI-RAMs. In addition, 4 PI-resistant, post-PI therapy samples contained changes near the CA/SP1 site, but retained susceptibility to GSK3532795.

The results of this study indicate that GSK3532795, a potent, once-daily, second-generation MI, maintains activity toward clinical isolates from PI-treated patients harboring baseline and/or progressive genotypic and phenotypic PI resistance. Emergent mutations in PR and Gag were not linked to reduced viral susceptibility to GSK3532795. A lack of cross-resistance of GSK3532795 to PI-resistant isolates with primary PI resistance supports the use of PIs and MIs simultaneously or in succession, and supports the continued development of GSK3532795 in treatment-experienced patients with previous PI treatment exposure.

ACKNOWLEDGMENTS

The authors thank Matthew Healy for sharing unpublished studies assessing the prevalence of MI-resistance mutations in PI-resistant viruses from the Los Alamos National Laboratory Database. Editorial support was provided by Sharmin Naaz at MediTech Media and funded by Bristol-Myers Squibb.

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