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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-IC_{50} (fold-change in IC_{50} versus wild-type virus)] <3 were considered to be within the no-effect level.

Results: All nonlongitudinal viruses tested were sensitive to GSK3532795 (FC-IC_{50} 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 (Monogram) 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

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INTRODUCTION

HIV and AIDS remain a global health issue despite the success of combination antiretroviral (ARV) therapy.[1] Lifelong 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

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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 Gag[13] 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 PI-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* 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 NL_{4-3} 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 NL_{4-3} 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 mutations[29] was analyzed for sensitivity to GSK3532795, BMS MI B, LPV, and ATV in the multiple-cycle assay. Isolates [NL_{4-3} background, PI-resistance mutations in *PR*, wild-type NL_{4-3} *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 NLRepRluc.

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 pHIVluc-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-IC_{50}) was determined by dividing the drug concentration leading to 50% viral inhibition (IC_{50}) values for the *Gag/PR* recombinant virus by those of a drug-sensitive reference virus containing the *Gag/PR* sequences of NL_{4-3}.

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^R Mutations in *PR*)		Predicted Resistance to PI (Stanford HIV Drug Resistance Database)							
			Major	Minor	ATV/r	DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r
Samples with reportable results in the Monogram assay												
pt02 pre-PI			None	L10I								
pt02 PTx 1	IDV, RTV, NFV, ATV, TPV	6.8	M46I, I84V, L90M	L10I, G73C								
pt02 PTx 2		11.2	M46I, I84V, L90M	L10I, G73C/S								
pt03 pre-PI			None	A71V								
pt03 PTx	NFV	3.1	L90M	A71V, G73G/S								
pt04 pre-PI			None	None								
pt04 PTx 1	SQV, RTV, NFV, LPV	5.7	M46L, G48V, I54V, V82A	K43T								
pt04 PTx 2		10.7	M46L, G48V, I54V, V82A	L10V, L24I, L33F, K43T								
pt06 pre-PI			None	L10I, A71T								
pt06 PTx 2	RTV, SQV, IDV	10.6	I54V, V82A, I84V	L10I, L23I, L24I, K43T, A71T								
pt07 pre-PI			None	None								
pt07 PTx	RTV, SQV	6	I54V, I84V, L90L/M	L10I/L, A71A/V								
pt09 pre-PI			None	L10I								
pt09 PTx 1	RTV, NFV	0.6	V32I, M46I, V82A	L10I, A71V								
pt09 PTx 2		4.2	V32I, M46I, V82A, L90M	L10I/V, L33F, A71V								
pt10 pre-PI			D30N	A71V								
pt10 PTx	IDV, NFV	5.9	D30N, M46I, I84V, L90M	L10I, A71V								
pt11 pre-PI			None	L10F/L, E35G								
pt11 PTx 1	NFV, LPV, SQV	3	M46L, I54V	L10F/I/L, E35D/G, N83D								
pt11 PTx 2		10	M46L, G48A, I54V, V82A	L10F/I/L, L33I, E35D/G/N, K43T, N83D								
pt12 pre-PI			None	L10L/V								
pt12 PTx	NFV, RTV	2	I54I/L, N88N/S	L10I/L/V								
pt14 pre-PI			None	None								
pt14 PTx	SQV, RTV	2.3	M46I/M, I84V, L90M	L10Y, A71V, G73S								
pt15 pre-PI			None	None								
pt15 PTx 1	SQV, RTV, LPV, APV, DRV	6.5	M46I, I54V, I84V, L90M	L10I, V11I/V, A71V, G73S								
pt15 PTx 2		11.7	M46I, I54V, V82C, I84V, L90M	L10I, V11I/V, K43T, A71V, G73S								
Samples re-tested using single- and multiple–cycle assays												
pt01 pre-PI			None									
pt01 PTx 1	SQV, RTV, NFV	5.8	M36V, G48V, V82A/V, L90M	L10F, A71V								
pt05 pre-PI			None	V77I								
pt05 PTx 1	RTV, SQV, NFV	5.4	M46L, L90M	G73S								
pt06 pre-PI			None	L10I, A71T								
pt06 PTx 1	RTV, SQV, IDV	5.3	I54V, V82A	L10I, L24I, A71T								
pt06 PTx 2		10.8	M36IM, I54V, V82A, I84V	L10I, L23I, L24I, E35D, K43T, A71T								
pt08 pre-PI			None	V77I								
pt08 PTx 1	NFV, LPV	3.1	D30N	A71V								
pt16 pre-PI			None	None								
pt16 PTx 1	RTV, IDV, NFV	6.9	M36I, M46L, I54V, V82A	L10I, L24I, A71V								

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^R, protease inhibitor resistance; PR, protease; PTx, post-PI treatment; pt, patient; lr, low-dose ritonavir; SQV, saquinavir; TPV, tipranavir.

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% CO_2, 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-IC_{50} 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

	FC-IC_{50}		CFB		*Gag* Genotype	
Sample Name	GSK3532795	DRV	GSK3532795	DRV	Gag PI^R Mutations	Gag Polymorphisms that May Affect MI Susceptibility[7,23,24,32]
Pt02 pre-PI	2.13	2.84				V370A, S373N, A374T, T375AN
Pt02 PTx 1	2.70	4.52	1.27	1.59		V370A, S373N, A374T, T375AN
Pt02 PTx 2	2.26	2.63	1.06	0.93		V370A, S373N, A374T, T375AN
Pt03 pre-PI	0.62	1.40				T375A, I376A
Pt03 PTx	0.39	1.37	0.63	0.98	A431V	T375A, I376A
Pt04 pre-PI	1.47	1.48				
Pt04 PTx 1	0.70	2.78	0.48	1.88		
Pt04 PTx 2	10.97	5.18	7.46	3.50	I473V, L449V	
Pt06 pre-PI	0.89	1.15				Q369H, T357I
Pt06 PTx 2	1.41	9.65	1.59	8.39	A431V, I437V, L449F	Q369H, N372H, delA374, T375I
Pt07 pre-PI	0.76	0.75				A374A, T375A
Pt07 PTx	0.30	2.17	0.39	2.89	A431V, P453L	S373P, A374S, T375A
Pt09 pre-PI	0.43	1.19				A374N, T375A
Pt09 PTx 1	11.88	3.49	27.45	2.93	A431V	V362I, A374N, T375A
Pt09 PTx 2	1.37	13.00	3.17	10.92	A431V	V362I, A374N, T375A
Pt10 pre-PI	0.54	1.45				S373P
Pt10 PTx	0.55	4.28	1.03	2.95	A431V, P453L	
Pt11 pre-PI	22.30	1.85			V128I	V370A, S373A, A374N, T375A
Pt11 PTx 1	54.96	2.64	2.46	1.43	K436R	V370A, S373A, A374N, T375A
Pt11 PTx 2	1.12	1.07	0.05	0.58	K436R	V370A, S373A, A374N, T375A
Pt12 pre-PI	0.24	0.67			L449P, P453L	A374S, T375A
Pt12 PTx	0.13	0.39	0.56	0.58	K436K/R	A374S, T375A
Pt14 pre-PI	256.82	1.02			L449P, P453L	
Pt14 PTx	58.36	2.00	0.23	1.96	L449P, P453L	
Pt15 pre-PI	1.09	0.79				G357S, A374S, T375A, I376V
Pt15 PTx 1	0.30	2.66	0.27	3.37	A431V, P453L	G357S, A374S, T375A, I376V
Pt15 PTx 2	0.52	4.14	0.48	5.24	A431V, P453L	G357S, delT371, A374S, T375A, I376V

CFB, change from baseline; DRV, darunavir; FC, fold-change; MI, maturation inhibitor; PI^R, protease inhibitor resistance; PTx, post-PI treatment; Pt, patient.

considered to be indicative of an effect on susceptibility.[31] 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-IC_{50} range 15–442) and ATV (FC-IC_{50} range 11–415), but retained susceptibility to GSK3532795 and BMS MI B, with FC-IC_{50} 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.[15] 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)[17–22] (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).[17,32] 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.[5,13,14,17,27,31,33–35] 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-IC_{50} 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-IC_{50} data not shown in Fig. 1). Although none of the samples had FC-IC_{50} >90, indicating clinically defined DRV resistance, the second post-PI treatment sample from Pt09 showed intermediate DRV resistance (FC-IC_{50} = 13). GSK3532795 susceptibility was observed in

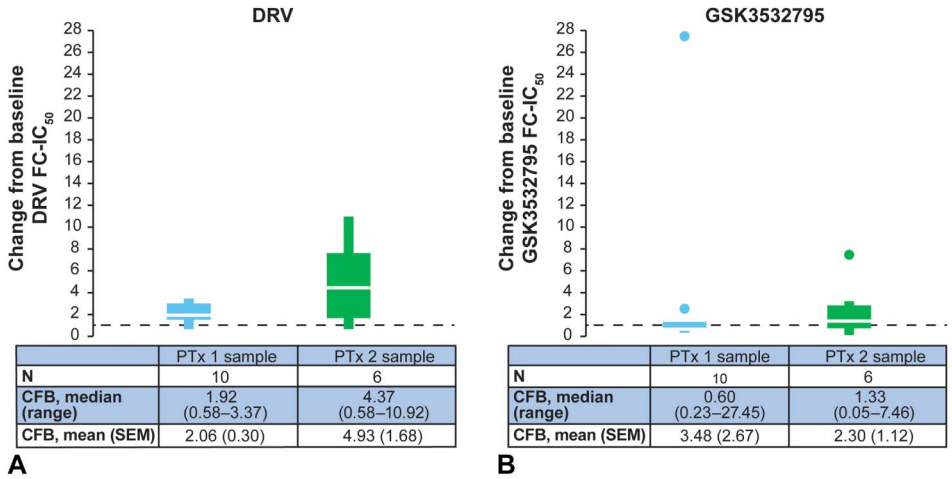


FIGURE 1. Longitudinal isolates (Monogram assay): change from baseline in (A) DRV and (B) GSK3532795 susceptibilities*. Pre- and post-treatment longitudinal samples were analyzed using the Monogram PhenoSense Gag/PR assay to determine their susceptibility to DRV and GSK3532795. CFB were calculated as a ratio of post-treatment and pre-treatment FC-IC_{50}s. For a subset of subjects, 2 sets of PTx samples were available. Median (range) and mean (SEM) of the CFB FC-IC_{50} for the 2 sets of PTx samples are shown in tables under each graph.*All posttherapy samples contain ≥1 major PI RAM (Monogram assay data). CFB, (FC-IC_{50} post-PI therapy/FC-IC_{50} pre-PI therapy); FC-IC_{50}, fold-change in IC_{50}; IC_{50}, drug concentration leading to 50% viral inhibition; PI, protease inhibitor; PTx, post-PI treatment; RAM, resistance-associated mutation; SEM, standard error of the mean.

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

	Samples Tested Using Gag/PR Pseudotype Single-Cycle Assays									
	CFB					FC-IC_{50}				
Sample Name	GSK3532795	BMS MI A	BMS MI B	ATV	LPV	GSK3532795	BMS MI A	BMS MI B	ATV	LPV
Pt01 pre-PI	1	1	1	1	1	0.90	0.33	0.41	1.4	1.3
Pt01 PTx	2	0.42	0.51	7.4	3	1.8	0.14	0.21	12.93	3.9
Pt05 pre-PI	1	1	1	1	1	2.9	2.8	1.4	0.9	0.2
Pt05 PTx	0.4	0.2	0.5	5.3	4.6	1.0	0.6	0.7	4.5	0.9
Pt06 pre-PI	1	1	1	1	1	1.1	0.44	0.71	1.0	1.2
Pt06 PTx 1	2.5	2.2	1.4	4.2	75.9	2.2	1.0	1.0	4.1	91.3
Pt06 PTx 2	1.4	0.92	0.87	3.4	18.5	1.5	0.41	0.61	3.4	22.3
Pt08 pre-PI	1	1	1	1	1	2.6	0.94	0.75	0.64	0.70
Pt08 PTx	0.49	0.2	0.36	2.9	1.1	1.3	0.19	0.27	1.7	0.74
Pt10 pre-PI	1	1	1	1	1	0.89	0.45	0.66	1.1	1.6
Pt10 PTx	0.83	0.85	0.60	4.5	4.0	0.68	0.39	0.40	4.8	6.2
Pt16 pre-PI	1	1	1	1	1	1.3	0.33	0.49	0.94	1.5
Pt16 PTx	1.7	1.1	1.8	28.9	31.2	1.8	0.35	1.0	26.2	48.0

	Samples Tested Using Gag/PR Pseudotype Single-Cycle Assays			
	PR Genotype (Primary PI^R Mutations in PR)		Gag Polymorphisms Relative to HIV-1 HXB2	
Sample Name	Major	Minor	Gag PI^R Mutations	Gag Polymorphisms in SP1 that May Affect MI Susceptibility[7,23,24,32]
Pt01 pre-PI	None	None		V370M, I376V
Pt01 PTx	M36V, G48V, V82A/V, L90M	L10F, A71V	I437V, P453L	V370M, I376V
Pt05 pre-PI	None	V77I		S373P, A374P, T375A
Pt05 PTx	M46L, L90M	G73S	A431V	S373P, A374P, T375A
Pt06 pre-PI	None	L10I, A71T		Q369H, T375N
Pt06 PTx 1	I54V, V82A	L10I, L24I, A71T	A431V, I437V	Q369H, T375N
Pt06 PTx 2	M361M, I54V, V82A, I84V	L10I, L23I, L24I, E35D, K43T, A71T	A431V, I437V, L449F	Q369H, N372H, delA374, T375N
Pt08 pre-PI	None	V77I		delT371, A374T
Pt08 PTx	D30N	A71V		A374S, delT371
Pt10 pre-PI	D30N			S373P
Pt10 PTx	D30N, M46I, I84V, L90M,		A431V, P453L	S373P
Pt16 pre-PI	None	None		V370M
Pt16 PTx	M36I, M46L, I54V, V82A	L10I, L24I, A71V	A431V	V370M

*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; PI^R, protease inhibitor resistance; PTx, post-PI treatment; pt, patient; RAM, resistance-associated mutation; SP1, spacer peptide-1.

9/11 pre-PI treatment samples, whereas low susceptibility to GSK3532795 was observed in the other 2 patients (FC-IC_{50} Pt11: 22.30; Pt14: 256.82). The determinants for reduced GSK3532795 susceptibility are currently being further examined in these samples and are not yet understood. However, the corresponding post-PI treatment samples had greatly reduced FC-IC_{50}, indicating enhanced susceptibility. Overall, although no consistent change was observed in the distribution of the GSK3532795 FC-IC_{50}s within this set, the distribution of the GSK3532795 FC-IC_{50}s 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 in patients 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 >3 (further analysis of these samples is presented in section "Further Analysis of Longitudinal Isolates With GSK3532795 CFB >3"). 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

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, Supplemental 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, Supplemental 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-IC_{50}: ATV = 402; LPV = 152; Pt09 time point 1 FC-IC_{50}: ATV = 14.8; LPV = 9.6) and the multiple-cycle assay (Pt04 time point 2 FC-IC_{50}: ATV = 217, LPV = 133; Pt09 time point 1 FC-IC_{50}: 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.

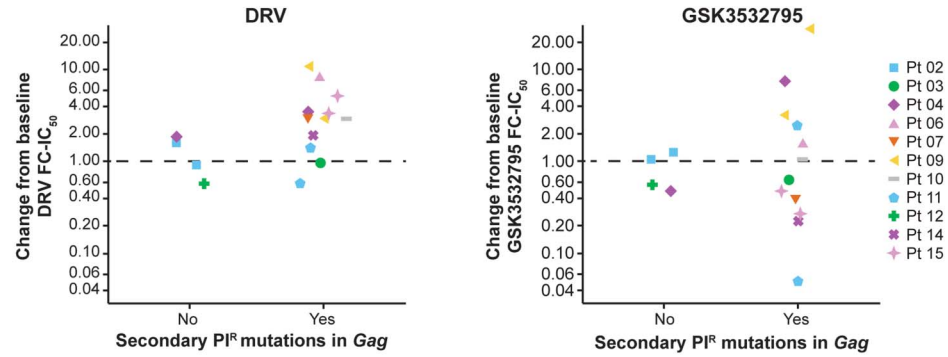
Impact of PI-Resistance Mutations in Gag Cleavage Sites on GSK3532795 Susceptibility

The most frequently observed mutations in the Gag polyprotein shown to affect PI susceptibility are in MA/CA (codon 128), NC/SP2 (codons 431, 436, and 437), and SP2/P6 (codons 449, 452, and 453).[17–22] None of these mutations map to amino acids associated with BVM susceptibility.[35] 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 Pt08) (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



Post-/pre-treatment FC-IC_{50}	No	Yes
N	4	12
Median (range)	1.26 (0.58, 1.88)	2.94 (0.58, 10.92)

A

Post-/pre-treatment FC-IC_{50}	No	Yes
N	4	12
Median (range)	0.81 (0.48, 1.26)	0.83 (0.05, 27.45)

B

FIGURE 2. Effect of Gag PI-resistance mutations on susceptibility to (A) DRV and (B) GSK3532795. Pre- and post-treatment longitudinal samples were analyzed using the Monogram PhenoSense Gag/PR assay to determine their susceptibility to DRV and GSK3532795. CFB were calculated as a ratio of the post-treatment and pretreatment FC-IC_{50}s. Posttreatment samples with and without secondary PI^R mutations in Gag were categorized into 2 groups with "Yes" and "No" flags, respectively. Median (range) values of the CFB FC-IC_{50} for the 2 groups are shown in tables under each graph. CFB, change from baseline; DRV, darunavir; FC-IC_{50}, fold-change in IC_{50}; IC_{50}, drug concentration leading to 50% viral inhibition; PI^R, protease inhibitor resistance; pt, patient.

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 sensitivity. As expected, viruses with reduced GSK3532795 susceptibility (FC-IC_{50} 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 MIs (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 Pt09 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 Pt04 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 Pt10 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 Pt04 and Pt09 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.

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