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Clinical evidence for a lack of cross-resistance between temsavir and ibalizumab or maraviroc

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Background: Temsavir (TMR), the active agent of the gp120-directed attachment inhibitor fostemsavir (FTR), the CD4-directed attachment inhibitor ibalizumab (IBA), and the CCR5 antagonist maraviroc (MVC) are antiretroviral agents that target steps in HIV-1 viral entry. Although mechanisms of inhibition of the three agents are different, it is important to understand whether there is potential for cross-resistance between these agents, as all involve interactions with gp120.

Methods: Envelopes derived from plasma samples from participants in the BRIGHTE study who experienced protocol-derived virologic failure (PDVF) and were co-dosed with FTR and either IBA or MVC were analyzed for susceptibility to the agents. Also, CCR5-tropic MVC-resistant envelopes from the MOTIVATE trials were regenerated and studies were performed to understand whether susceptibility to multiple agents were linked.

Results: The cloned envelopes exhibited reduced susceptibility to TMR and resistance to the co-dosed agent. At PDVF, emergent or preexisting amino acid substitutions were present at TMR positions of interest. When amino acid substitutions at these positions were reverted to the consensus sequence, full susceptibility to TMR was restored without effecting resistance to the co-dosed agent. In addition, five envelopes from MOTIVATE were regenerated and exhibited R5-tropic-MVC-resistance. Only one exhibited reduced susceptibility to TMR and it contained an M426L polymorphism. When reverted to 426M, full sensitivity for TMR was restored, but it remained MVC resistant.

Conclusion: The data confirm that decreased susceptibility to TMR and resistance to IBA or MVC are not linked and that there is no cross-resistance between either of these two agents and FTR.

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Introduction

As treatment of HIV-infection involves co-dosing with multiple (two or more) antiretroviral agents, it is important to understand the resistance mechanisms for each agent. If an emergent mutation pathway can result in decreased susceptibility to a co-dosed agent, regardless of the target, then co-dosing with these drugs should be avoided. Fostemsavir (FTR), the prodrug of temsavir (TMR), is a first-in-class gp120-directed attachment inhibitor indicated for heavily treatment-experienced (HTE) adults with multidrug-resistant HIV-1 failing their current antiretroviral regimen due to resistance, intolerance, or safety considerations [1–3]. Temsavir binds in a pocket around the $CD4^+$-binding site in gp120 and is believed to stabilize the protein in a 'closed' conformation

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that is unable to bind to $CD4^+$ [4–6]. Present studies show that reduced susceptibility to TMR in the clinic maps to specific changes at four amino acid positions (375, 426, 434, and 475) surrounding the binding site of TMR [3,7–9].

IBA and MVC are two other approved anti-HIV medications that target viral entry, albeit at different steps compared with TMR. IBA is a mAb that binds to host $CD4^+$ receptor protein and inhibits gp160 conformational changes required for virus-cell fusion through steric hindrance [10–13]. Resistance to IBA maps to loss of N-linked glycosylation sites in the V5 region of gp120 [14,15]. Maraviroc is a small molecule that binds to the host CCR5 protein and inhibits gp160 binding to this co-receptor [16]. Given this mechanism of action, MVC is only active against the CCR5-tropic subset of HIV viruses and has little to no activity against CXCR4-tropic or dual-tropic viruses. Resistance of CCR5-tropic viruses to maraviroc map to changes within the V3 loop of gp120 that binds to CCR5 [16]. Thus, given that TMR, IBA, and MVC all target viral entry, albeit by different mechanisms and at different regions of gp120, it is imperative to understand whether certain sequence changes can induce cross-resistance of TMR to IBA or MVC.

Previous in-vitro studies have suggested that TMR lacks cross-resistance with either IBA or MVC. Both IBA and MVC exhibited full activity against envelopes from clinical samples that contained changes in gp120 that had produced reduced susceptibility to TMR [17]. Also, an NL_{4-3} virus selected in cell culture to have increased resistance to IBA retained sensitivity to TMR. However, clinical envelopes with resistance to IBA had not been tested. For maraviroc, only limited data have been available. Some CCR5-tropic MVC-resistant envelopes available did exhibit reduced susceptibility to TMR, while others had full susceptibility to TMR [17]. Therefore, more defined data from clinical studies are needed to confirm these initial findings. In this study, clinical samples from the BRIGHTE and MOTIVATE [3,18,19] Phase 3 studies were used to conclusively show the lack of cross-resistance between TMR and these agents.

Materials and methods

The Materials and Methods are provided in Supplementary Information, http://links.lww.com/QAD/C335.

Results

Lack of cross-resistance between temsavir and ibalizumab

Initially, only five cloned ibalizumab-resistant envelopes from different individuals could be identified in the Monogram BioSciences library for testing. The five clonal samples were analyzed in a PhenoSense Entry Assay for susceptibility to both TMR and ibalizumab. The results are summarized in Table 1.

Table 1. Susceptibility of TMR and ibalizumab against envelopes in the PhenoSense Entry Assay.

Samples from Monogram						
Sample ID	TMR IC_{50} (nmol/l)	TMR IC_{50}-Fold change	TMR MPI (%)	IBA IC_{50} (μg/ml)	IBA IC_{50}-Fold change	IBA MPI (%)
E08_154037_12	>5	>MAX	45	0.749742	31	62
E08_154024_12	0.014782	13	100	0.594808	24	60
E08_134227_16	0.013569	12	100	0.035088	1.45	86
E08_177370_14	1.619040	1458	74	0.211326	8.7	67
DUAL	0.000981	0.88	100	0.054636	2.25	84

Samples from BRIGHTE							
Sample ID	Visit	TMR gp120 polymorphisms	TMR IC_{50} (nmol/l)	TMR IC_{50}-Fold change	IBA IC_{50} (μg/ml)	IBA IC_{50}-Fold change	IBA MPI (%)
Individual 153	Screening	M426L	96.28	100.00	0.015029	0.62	99
	Week 13 (PDVF)	M426L	87.34	99.00	0.093108	3.83	72
Individual 336	Screening	S375T	10.89	16.00	0.016563	0.68	100
	Week 108 (post PDVF)	S375T M426L	>5000	>3324.51	0.068232	2.81	72
Individual 508	Screening	none	0.47	0.55	0.022852	0.94	93
	Week 36 (PDVF)	S375H/N M426M/L	>5000	>3651.39	0.069740	2.87	74
Individual 552	Screening	M426M/L	0.39	0.43	0.017847	0.74	98
	Week 74 PDVF	NR	NR	NR	ND	ND	ND
Individual 559	Screening	M426M/T	0.89	0.98	0.016481	0.68	64
	Week 6 (pre-PDVF)	S375N	253.94	223	0.198098	8.16	51
	Week 36 (PDVF)	S375N M475I	>5000	>2500	1.486080	61.00	62

FC, fold change.

All five samples exhibited decreased susceptibility to ibalizumab based upon a low maximum percentage inhibition (MPI; 60–87%). In addition, the clones with the lowest MPI correlated with higher IBA IC_{50}-FCs, with MPIs of 60, 62, and 67% and IBA IC_{50}-FCs of 8.7, 24, and 31, respectively. Of these five clones, three exhibited excellent susceptibility to TMR (E08_154024_12, E08_154227_12, and DUAL), with low IC_{50}s and IC_{50}-FCs. These three clones also exhibited MPIs of 100% against TMR. The other two clones were not readily susceptible to TMR, with IC_{50}s of nearly 1.6 and more than 5 μmol/l (TMR IC_{50}-FCs = 1458 and >maximum, respectively) and TMR MPIs of 74 and 45%, respectively. Although this and previous work [17] suggest that resistance to the two molecules is not linked, additional work was initiated.

The BRIGHTE (NCT02362503) study is an ongoing phase 3 study investigating the efficacy and safety of fostemsavir as well as optimized background therapy (OBT) in highly treatment-experienced (HTE) individuals who were failing their current regimen (confirmed HIV-1 RNA ≥400 copies/ml) with limited remaining antiretroviral treatment options [2].

BRIGHTE enrolled in two distinct cohorts (Supplemental Figure 1, http://links.lww.com/QAD/C335). In the randomized cohort, participants were enrolled who had at least one but no more than two antiretroviral classes remaining at study entry and could not form a viable antiretroviral regimen out of available fully active agents. FTR (600 mg) twice daily (b.i.d.) was administered on top of the current failing regimen for 8 days of functional monotherapy and then participants were switched to an OBT with FTR. In the randomized cohort, there were no participants who received IBA as part of their initial OBT. The non-randomized cohort consisted of participants without any remaining fully active and approved antiretroviral agents (Supplemental Figure 1, http://links.lww.com/QAD/C335). All participants in the non-randomized cohort received FTR 600 mg b.i.d. with OBT on Day 1. There were 15 participants in the non-randomized cohort who received IBA therapy as part of their initial OBT.

Through Week 96, five of these 15 participants met the criteria for protocol-defined virologic failure (PDVF) and plasma samples at baseline and PDVF were available for four of these participants. The data from the Monogram PhenoSense Entry Assay and population sequencing at the four key TMR amino acid positions (S375, M426, M434, M475) from the four participants with Screening and PDVF samples are summarized in Table 1. At screening, one individual (Individual 153) exhibited a slightly high IC_{50} against TMR at screening (~96 nmol/l) and contained a key polymorphism of M426L. The other participants either had no key polymorphisms, minor polymorphs (S375T) or mixtures (M426 M/T) but were susceptible to TMR at baseline (0.39–11 nmol/l). All five participants were susceptible to IBA at screening based upon IC_{50} (0.015–0.023 μg/ml), but one individual (Individual 559) exhibited a reduced MPI of 64% at screening, indicative of IBA resistance. In the PDVF samples, three participants (Individuals 336, 508, and 559) had emergent substitutions at key amino acid positions and greatly reduced susceptibility to TMR. The other individual (Individual 153) contained the M426L polymorphism at screening and did not have any emergent mutations at the other three positions. This individual's susceptibility to TMR did not change at PDVF (IC_{50}-FC = 99). At failure, all four samples exhibited low MPI (51–72%) against IBA, with all showing at least a modest change in IBA IC_{50}-FC (2.81–61). Thus, these four participants exhibited low susceptibility to both TMR and IBA at PDVF.

A separate set of plasma samples from the three BRIGHTE participants with decreased susceptibility to both TMR and ibalizumab at PDVF (Individuals 508, 559, 336) were used to obtain RNA and their envelope genes were amplified and cloned. The sequences of a functional clone from each sample are shown in Supplemental Figure 2, http://links.lww.com/QAD/C335. Each clone contained either two key TMR changes at amino acid positions 375 and 426 (S375T or S375N along with M426L for Individuals 336 and 508, respectively) or a single M475I mutation [Individual 559; the emergent S375N mutation observed in the population sequence (Table 1) was not present in this clone] (Table 2). Each of these three envelopes exhibited a TMR IC_{50} more than 2 μmol/l in a phenotype assay. Each envelope also exhibited resistance to IBA, with the three clones exhibiting a low MPI, while the clone from Participant 559 also exhibits a high IC_{50} to ibalizumab (>800 nmol/l) (Table 2). Using these clones, site-directed mutagenesis was performed to mutate the known TMR substitutions back to their consensus amino acid sequence (T/N375S, L426 M, and I475 M). Analysis of susceptibility of all these clones to both TMR and ibalizumab is summarized in Table 2. When amino acids positions (375/426 or 475) were mutated back to wild type, sensitivity to TMR of all three cloned envelopes in a phenotype assay approached screening levels and were highly sensitive (TMR IC_{50} between 1 and 10 nmol/l). On the contrary, these changes in the three envelope clones had no effect on sensitivity or MPI for ibalizumab. As an internal control, there was no change in the susceptibility to raltegravir with any of the envelope clones.

In a separate experiment, there was one clone (pNL/SV40/gp160/PT336-WK108) wherein a part of the V5 region (KTVTRSNN) in the PDVF sample was replaced with the V5 region found in the Screening clone from this individual (HNTTNNSNK; pNL/SV40/gp160/PT336-WK108-V5 edited). When susceptibilities of this

Table 2. Antiviral activity against single clones of PDVF samples and site-directed mutants from BRIGHTE.

	TMR polymorphism	Temsavir IC_{50} (nmol/l)	Raltegravir IC_{50} (nmol/l)	Ibalizumab IC_{50} (nmol/l)	Ibalizumab MPI (%)
Individual 336 Wk 108	S375T and M426L	>2000	5.41 ± 0.78	2.39 ± 1.14	71 ± 1.7
Individual 336 Wk 108 T375S,L426M [a]	none	10.72 ± 2.07	4.75 ± 0.83	2.66 ± 0.53	69 ± 2.5
Individual 508 Wk 36	S375N and M426L	>2000	4.57 ± 0.93	1.56 ± 0.16	87 ± 2.0
Individual 508 Wk 36 N375S,L426M[a]	none	1.16 ± 0.57	5.54 ± 1.03	1.47 ± 0.12	64 ± 18.6
Individual 559 Wk 36	M475I	>2000	4.75 ± 1.01	>800	38 ± 15.9
Individual 559 Wk 36 I475M[a]	none	5.61 ± 2.01	6.16 ± 2.36	>800	25 ± 0.9
pNL/SV40/gp160/ PT336-WK108	S375T and M426L	>1000	Not done	0.56 ± 0.26	76 ± 8.23
pNL/SV40/gp160/ PT336-WK108-V5 edited	S375T and M426L	>1000	Not done	0.26 ± 0.45	93 ± 1.74

[a]Clones where known TMR substitutions are reverted to wild-type sequence.

envelope were compared with the PDVF clone, there was no change in TMR sensitivity, while the IBA IC_{50} showed little change and was relatively low but the MPI was raised from 76 to 93%. Thus, there was rescue of ibalizumab sensitivity in this clone without affecting TMR potency.

Lack of cross-resistance between temsavir and maraviroc in participants from BRIGHTE

Analysis of BRIGHTE results were performed in two different ways (Supplemental Figure 1, http://links.lww.com/QAD/C335). For one, there was an analysis of the effect of FTR on virologic outcome in participants in the randomized cohort who entered the study on a failing regimen that included MVC and received FTR during the initial 8-day functional monotherapy study. Excluding participants with evidence of possible residual activity of the failing regimen, there were 18 participants experiencing virologic failure on an MVC-containing regimen and of those, 15 (83%) experienced virologic success (>−0.5 log_{10} HIV-1 RNA) at Day 8 of FTR therapy. The results are summarized in Table 3. All eight (100%) participants with CCR5-tropic virus experienced virologic success, while zero out of two (0%) participants with CXCR4-tropic and seven out of eight (88%) participants with Dual-mixed virus experienced virologic success at Day 8. Previously, it was shown that tropism did not affect the susceptibility of envelopes to TMR [7,20].

Table 3. Viral load drops on Day 8 from participants who failed a MVC-containing regimen in BRIGHTE[a].

Participant	Tropism	Day 8 Success >0.5 log_{10} c/ml change in HIV-1 RNA from Day 1 to Day 8.	Change in HIV-1 RNA from Day 1 to Day 8 (log_{10} c/ml)	Baseline susceptibility to TMR (nmol/l)
529	CCR5	Y	−0.942	6.38
588	CCR5	Y	−0.700	1.99
794	CCR5	Y	−1.778	0.07
203	CCR5	Y	−0.785	9.27
393	CCR5	Y	−1.921	>5000
238	CCR5	Y	−0.753	0.25
150	CCR5	Y	−1.235	0.37
139	CCR5	Y	−2.020	19.18
008	DM	N	0.048	0.11
086	DM	Y	−1.080	0.56
163	DM	Y	−1.248	1.60
080	DM	Y	−2.696	0.45
674	DM	Y	−1.513	0.25
169	DM	Y	−1.387	0.43
130	DM	Y	−1.336	19.41
152	DM	Y	−1.311	2.19
483	CXCR4	N	−0.434	15.94
082	CXCR4	N	−0.187	31.36

[a]Day 8 population excludes participants with baseline HIV-1 RNA < 1000 c/ml and/or change from screening to baseline of HIV-1 RNA >0.3 log_{10} c/ml.

Table 4. Antiviral activity of TMR and MVC in Participant samples and single clones of Screening, PDVF samples, and site-directed mutants from BRIGHTE.

Participants	Sample type	TMR polymorphisms	TMR IC_{50} (nmol/l)	MVC IC_{50} (nmol/l)	MVC MPI (%)
474	Screen: Population	M426L	66	ND	ND
	PDVF: Population	S375N, M426L	372	ND	ND
474	Screening clone	M426L	234.1	135.9	88.8
	PDVF clone	M426L	788.9	89.6	93.5
	Screening clone SDM	none	1.1	169.5	91.1
	PDVF clone SDM	none	1.3	166.2	93.8
203	Screen: Population	M434T	9	ND	ND
	PDVF: Population	S375N, M426L, M434T	>5000	ND	ND
203	Screening clone	M434T	69.7	>5000	−13.9
	PDVF clone	M426L, M434T	>2000	>5000	−2.6
	Screening clone SDM	none	2.2	>5000	6.7
	PDVF clone SDM	M434T	432.2	>5000	−7.3
	PDVF clone SDM	M426L	171.3	>5000	−2.2
	PDVF clone SDM	none	5.9	>5000	−8.1

ND, not done.

In a second initiative, samples from two CCR5-tropic virus-infected participants co-dosed with both FTR and MVC during BRIGHTE who experienced PDVF through Week 96 were analyzed. MVC sensitivity data were not collected during the study (only tropism information was collected), so all MVC susceptibility work was performed with envelope clones. In terms of TMR and the population, Individual 474 possessed M426L at baseline and exhibited a slightly elevated IC_{50} (66 nmol/l) to TMR, with S375N emergent at PDVF along with M426L, resulting in a TMR IC_{50} of 372 nmol/l. Individual 203 possessed M434T at baseline with a TMR IC_{50} of 9 nmol/l and had M426L and S375N emerge at PDVF alongside M434T (Table 4).

Separate plasma samples were then processed in-house and functional clones of the env gene from Screening and PDVF samples from both participants were made and tested against TMR and MVC. Table 4 summarizes that the clones obtained from the Screening and PDVF samples from both participants mirrored the population data in susceptibility, although S375N observed in both population sequences was not present in the clonal sequence. Site-directed mutagenesis was then performed to revert the TMR-associated changes back to wild-type sequences (426 M, 434 M). The data (Table 4) clearly show that susceptibility to TMR and MVC is not linked in these samples, as reverting the TMR-associated amino acids increases susceptibility to TMR without affecting MVC susceptibility.

Activity of temsavir against envelopes from MOTIVATE

The MOTIVATE 1 and 2 studies were registrational Phase 3 studies that were used for approval of maraviroc for use in CCR5-tropic HIV-1 infected individuals [19]. In an effort to analyze the potency of TMR against CCR5-tropic-virologic failure samples from MOTIVATE studies, a series of envelopes was regenerated. Only the gp120 sequences of these CCR5-tropic envelopes were available in GenBank, so full-length clones were regenerated by addition of a gp41 sequence from a full-length gene using gp41 sequences from full-length genes in GenBank wherein gp120 gene was closest in homology to the participant gp120. The GenBank accession numbers of the various gp120 and gp41 sequences that were used are found in Supplementary Table 1, http://links.lww.com/QAD/C335. Five individual envelope genes were synthesized, with one of the genes containing a baseline M426L polymorphism (MP11.38). Therefore, a sixth envelope was synthesized that was identical to MP11.38 except for an L426 M mutation (MP11.38 (L426 M)).

All five of the original envelopes were CCR5-tropic (based upon inactivity of the CXCR4 antagonist AMD3100 [21]) and exhibited high levels of resistance to MVC, with IC_{50} values more than 5 μmol/l for all envelopes (Table 5). Of these five gp160 s, four were highly susceptible to TMR, with IC_{50} values between 0.5 and 1.5 nmol/l. The outlier was the M426L-containing

Table 5. Susceptibility of MOTIVATE-derived CCR5-tropic MVC-resistant virus envelopes to TMR.

Envelope	TMR IC_{50} (nmol/l)	MVC EC_{50} (nmol/l)	MVC MPI (%)	AMD3100 EC_{50} (nmol/l)
MP5.7	1.00 ± 0.40	>5000	<0	>6000
MP35.2	1.00 ± 0.28	>5000	5.41 ± 13.99	>6000
MP49.20	1.56 ± 0.67	>5000	<0	>6000
MP53.36	0.51 ± 0.05	>5000	<0	>6000
MP11.38	412.70 ± 44.66	>5000	<0	>6000
MP11.38 (WT426)	0.98 ± 0.35	>5000	6.38 ± 7.88	>6000

MP11.38 envelope, with an IC_{50} of nearly 413 nmol/l. However, when residue 426 was reverted to methionine, the IC_{50} to TMR also reverted to a highly sensitive nearly 1 nmol/l without affecting MVC susceptibility, again showing the lack of cross-resistance of these agents.

Discussion

Reduced susceptibility to FTR treatment in clinical envelopes has been mapped to four positions in gp120. Thus, certain changes at S375, M426, M434, and M475 tend to decrease susceptibility to TMR and can emerge during FTR treatment [7]. These amino acids surround the binding site of TMR and reduced susceptibility can be explained through modeling interactions [4,6]. These emergent mutations can also be present as preexisting polymorphisms in a percentage of virus envelopes, although the large majority of baseline envelopes remain susceptible to TMR, as 91% of 1337 envelopes examined in a PhenoSense assay exhibited IC_{50} values less than 100 nmol/l [20]. An important exception to the broad coverage of TMR is with CRF01_AE viruses, wherein envelopes routinely exhibited high IC_{50} values to TMR, owing to the regular presence of two key polymorphisms (S375H and M475I) in most CFR01_AE viruses [7,20]. However, these known polymorphisms present in untreated viruses could explain why some clinical envelopes that were not exposed to TMR can show reduced susceptibility. This can be observed within the cohort of five cloned envelopes that were ibalizumab-resistant and tested at Monogram BioSciences (Table 1), where two envelopes also exhibited reduced susceptibility to TMR. Also, previous work with two CCR5-tropic MVC-resistant envelopes showed that one envelope exhibited reduced susceptibility to TMR, while the other did not [7]. Although additional data suggested that TMR showed a lack of cross-resistance to both IBA and MVC, further evaluation was needed to confirm these observations, preferably using clinical isolates from participants co-dosed with these agents.

The BRIGHTE study provided just such a platform to further investigate the potential of cross-resistance between TMR and other entry inhibitors. BRIGHTE was a registrational Phase 3, international, double-blind, placebo-controlled trial that evaluated the efficacy and safety of fostemsavir (RUKOBIA) in patients with HIV-1 who were HTE with limited treatment options remaining [2,3]. In this study, there were participants who were co-dosed with FTR and IBA or MVC, who met the criteria for PDVF and exhibited decreased susceptibility or resistance to the two agents. It should be noted that only one sample (PDVF or closest to it) was analyzed, so that no information on sequential mutations under drug treatment was obtained. Through clonal analysis and site-directed mutations, it was shown that susceptibility to one agent can be changed without affecting susceptibility to the other. In all cases, decreased susceptibility to TMR could be traced to an emergent change at residues 375, 426, or 475 or a preexisting polymorphism. Preexisting polymorphisms that affected TMR susceptibility were identified in three participants; one co-dosed with IBA (Individual 153 with M426L at Screening) and two with MVC (Individuals 203 and 474 with M426L and M434T at Screening, respectively). The M434T polymorphism is extremely rare, with 32 of 7561 (0.4%) full length envelopes in the Los Alamos National Laboratory (LANL) database of HIV sequences having this change, which results in a 15-fold drop in susceptibility when tested in an LAI envelope background [7]. Within the six participants (where data were available) who encountered PDVF when co-dosed with FTR and either IBA or MVC, five participants had emergent substitutions of S375H/N, M426L, or M475I, while Individual 153, with an M426L at Screening, had no TMR-specific emergent changes but did exhibit a relatively high but unchanged IC_{50} at Screening and PDVF. In all cases though, reverting the TMR-specific substitution to the wild-type residue greatly increased the susceptibility to TMR while not affecting resistance to either IBA or MVC. Also, in a clone from Individual 336, reverting the V5 region in the PDVF sample to that found in the Screening clone from this individual increased the MPI for IBA without affecting TMR susceptibility. This strongly supports the notion that the two viral entry inhibitors (IBA and MVC) exhibit no intrinsic cross-resistance to TMR. This might have been expected given the disparate resistance profiles of the three entry inhibitors, with resistance to IBA mapping to the loss of PNGSs in the V5 loop [14], resistance to MVC mapping to changes in the V3 loop [16], and reduced susceptibility to TMR mapping to defined amino acids around the $CD4^+$-binding site, where TMR binds [4,6,7]. However, it has been shown that for TMR, changes far away from the binding site, at L116P and A204D in the LAI envelope, can greatly reduce susceptibility to TMR [7], even if these changes have not been observed in the clinic and are extremely rare in the LANL database of envelope sequences [22]. Thus, it is important to evaluate clinical samples to confirm the lack of cross-resistance.

Additional data illustrating the lack of cross-resistance with TMR and MVC were also available. As several participants in BRIGHTE entered the study with MVC included in their failing regimen, one can examine whether addition of FTR in this cohort was successful. Out of 18 participants in the Randomized cohort experiencing virologic failure on a MVC containing regimen, 15 (83%) experienced virologic success, as defined by a viral load drop at Day 8 of more than $0.5\log_{10}$ copies/ml and median viral load reduction in these 18 participants was 1.2 log10 copies/ml. This is actually better than in the BRIGHTE study as a whole

where, among FTR-treated participants in the randomized cohort who had a baseline HIV-1 RNA more than 1000 copies/ml, 68% of participants achieved viral load reduction of more 0.5 log10 copies/ml and median decrease in HIV-1 RNA from baseline to day 8 was 1.02 log10 copies/ml [2].

Of the three virologic failures in the cohort of 18, two were CXCR4-tropic, so MVC would have had no activity as part of the failing regimen, and the third was a Dual Mixed virus. All eight individuals with CCR5-tropic virus achieved virologic success at Day 8, indicating that if failure on an MVC-containing regimen is used as a marker for MVC resistance, then MVC resistance does not prevent success with FTR. Previously, it has been shown that TMR is tropism agnostic and has similar activities against CCR5, CXCR4, and Dual mixed-tropic viruses [7,20]. In addition, reconstituted envelopes from MOTIVATE were generated and four of five (80%) exhibited excellent susceptibility to TMR, while five of five were CCR5-tropic, MVC resistant. The lone envelope with a reduced IC_{50} to TMR contained the known M426L TMR polymorphism. When this residue was converted to 426 M, full susceptibility to TMR was regained without affecting the MVC profile. This is further evidence of the lack cross-resistance of TMR and MVC.

Although the numbers of analyzed samples are relatively low, the results presented herein support the use of FTR in participants co-dosed with IBA or MVC, or in participants who have already failed treatment with either entry inhibitor, as the data show there is no intrinsic cross-resistance between TMR and these other two entry-targeted agents.

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Conflicts of interest

R.R., M.G., Z.L., N.A., M.C., J.B., M.L., P.A., and M.K. are employees of ViiV Healthcare and may own stock in GlaxoSmithKline.

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