The Journal of Infectious Diseases

MAJOR ARTICLE

Drug Resistance Assessed in a Phase 3 Clinical Trial of Maribavir Therapy for Refractory or Resistant Cytomegalovirus Infection in Transplant Recipients

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Background. This drug resistance analysis of a randomized trial includes 234 patients receiving maribavir and 116 receiving investigator-assigned standard therapy (IAT), where 56% and 24%, respectively, cleared cytomegalovirus DNA at week 8 (treatment responders).

Methods. Baseline and posttreatment plasma samples were tested for mutations conferring drug resistance in viral genes *UL97*, *UL54*, and *UL27*.

Results. At baseline, genotypic testing revealed resistance to ganciclovir, foscarnet, or cidofovir in 56% of patients receiving maribavir and 68% receiving IAT, including 9 newly phenotyped mutations. Among them, 63% (maribavir) and 21% (IAT) were treatment responders. Detected baseline maribavir resistance mutations were *UL27* L193F (n = 1) and *UL97* F342Y (n = 3). Posttreatment, emergent maribavir resistance mutations were detected in 60 (26%) of those randomized to maribavir, including 49 (48%) of 103 nonresponders and 25 (86%) of the 29 nonresponders where viral DNA initially cleared then rebounded while on maribavir. The most common maribavir resistance mutations were *UL97* T409M (n = 34), H411Y (n = 26), and C480F (n = 21), first detected 26 to 130 (median 56) days after starting maribavir.

Conclusions. Baseline maribavir resistance was rare. Drug resistance to standard cytomegalovirus antivirals did not preclude treatment response to maribavir. Rebound in plasma cytomegalovirus DNA while on maribavir strongly suggests emerging drug resistance.

Clinical Trials Registration. NCT02931539.

Keywords. antiviral drug resistance; antiviral therapy; cytomegalovirus; maribavir.

For a long time, the only approved systemic antiviral drugs for treatment of human cytomegalovirus (CMV) infections consisted of ganciclovir, its prodrug valganciclovir, foscarnet, and cidofovir, all targeting the viral DNA polymerase. Recently, maribavir was approved for treatment of CMV infections refractory (with or without resistance) to standard therapy. After promising phase 2 trials [1, 2], a phase 3 randomized trial showed that 56% of 234 patients who received maribavir and 24% of 116 receiving investigator-assigned standard therapy (IAT, chosen from the polymerase inhibitors listed above) achieved confirmed plasma viral DNA clearance at study week 8 and were classified as treatment responders [3]. This study involved patients at higher risk for adverse treatment outcomes, who were refractory to prior therapy, and the majority of whom had baseline genotypic data showing a known drug resistance mutation (DRM). Maribavir treatment avoided the hematologic toxicity of ganciclovir and the nephrotoxicity of foscarnet, both of which contributed to a higher rate of early discontinuation of therapy and impact on response rates [3].

Maribavir is a potent and selective benzimidazole L-riboside inhibitor of the CMV *UL97* kinase [4] that has multiple

Received 31 May 2023; editorial decision 21 July 2023; accepted 27 July 2023; published online 28 July 2023

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The Journal of Infectious Diseases® 2024;229:413–21

https://doi.org/10.1093/infdis/jiad293

activities important for viral replication [5, 6]. Knockout of *UL97* kinase activity severely reduces but does not eliminate viral growth [5]. Another activity of the *UL97* kinase is the initial phosphorylation of ganciclovir needed for generation of its triphosphate as a CMV DNA polymerase inhibitor [7]. Mutations in the *UL97* gene that impair ganciclovir phosphorylation with little impact on viral replication have long been known to be the principal mechanism of CMV ganciclovir resistance [8]. The most common of these mutations, at codons 460, 520, and 591–607, do not overlap the *UL97* mutations that commonly confer maribavir resistance, such as substitutions T409M and H411Y described in vitro before late-stage clinical trials were conducted [9]. However, more recent phase 2 trials have shown ganciclovir-maribavir cross-resistance arising in vivo resulting from the *UL97* substitutions F342Y and C480F [10]. Because resistance to the newer CMV antivirals letermovir (approved for use as prophylaxis [11]) and maribavir appears to be readily selected in vitro [10, 12, 13], their incidence of drug resistance in clinical use requires attention.

Importantly, the previous report on maribavir resistance during phase 2 trials highlighted the prominence of *UL97* substitutions T409M, H411Y, and C480F, and a rebound of plasma CMV during maribavir therapy as indicators of emerging resistance [10]. This analysis of genotyping data from a phase 3 randomized trial expands the earlier information and provides a comparison with the baseline and emergent resistance mutations for maribavir and the standard CMV DNA polymerase inhibitor drugs.

METHODS

Study Population, CMV Treatment and Primary End Point

As previously published [3], this phase 3 trial (SOLSTICE, NCT02931539) enrolled 352 solid organ or hematopoietic cell transplant recipients with CMV infection resistant or refractory to treatment with standard therapy, who were randomized to receive either maribavir or IAT for 8 weeks in a 2:1 ratio. As part of the phase 3 trial, all patients/legal guardians provided written informed consent, and the trial was approved by the institutional review board of each participating institution. The resistance analysis study population consisted of the 350 patients who received at least 1 dose of study drug (234 maribavir and 116 IAT). Of those receiving IAT, the investigator-assigned drug was foscarnet (n = 47), ganciclovir (n = 28), valganciclovir (n = 28), foscarnet combined with ganciclovir or valganciclovir (n = 7), or cidofovir (n = 6) [3].

The primary end point (criterion for treatment responder) was defined as clearance of plasma CMV DNA to <137 IU/mL (lower limit of detection) in consecutive samples obtained at least 5 days apart, when assessed at the end of study week 8. CMV DNA quantitation was done in a central laboratory using the COBAS AmpliPrep/COBAS TaqMan assay (Roche diagnostics). Nonresponders included those who had missing virologic data at week 8, usually because of early discontinuation of study drug, or who were transitioned to alternative therapy before this time point, including 22 receiving IAT who were switched to maribavir (rescue therapy). Those who initially cleared their plasma CMV DNA but had a rebound of viral DNA at the 8-week timepoint were also classified as nonresponders [3].

Resistance Analysis Protocol

Baseline genotypic analysis was a scheduled event, along with repeated genotypic analysis for plasma CMV DNA >455 IU/mL at weeks 4, 8 (end of treatment), 16, and 20 (follow-up period), as well as with discontinuation of therapy or rebound of viral load after previous clearance or nadir load at least 1 log lower. Resistance genotyping was performed by a central laboratory on DNA extracts of frozen plasma samples by nested polymerase chain reactions (PCRs) and fluorescent dideoxy (Sanger) sequencing [14] using primers providing overlapping sequence coverage of the entire viral genes *UL97*, *UL54,* and *UL27*. Sequences were aligned with those of the reference strain AD169. Automated readouts of amino acid substitutions were rechecked by visual inspection of sequencing chromatograms in cases of novel appearance of previously unknown mutations, or mixed sequence populations in low copy number specimens (<1000 IU/mL). Readouts with low quality scores or lacking confirmatory chromatograms were rejected [10, 14]. Detected amino acid substitutions were classified as known DRMs documented by recombinant phenotyping data [8], presumptive resistance mutations based on unpublished phenotype data or other known resistance mutations at the same codon locations, sequence polymorphisms previously observed in untreated baseline specimens, and variants of unknown phenotype. Sequencing data for each gene were considered incomplete if information was missing for codon ranges known to be involved in drug resistance.

For purposes of this study, all counts of DRMs include phenotypically confirmed resistance mutations, based on the number of patients (n) in whom they were detected. Tallies of maribavir-DRMs or IAT-DRMs (mutations conferring resistance to ganciclovir, cidofovir, and/or foscarnet) include the counting of maribavir-ganciclovir dual-resistant mutations *UL97* F342Y and C480F in both categories, and IAT-DRM counts include those for any of the licensed CMV DNA polymerase inhibitors regardless of the specific IAT drug assigned for an individual case. Treatment-emergent mutations were those that appeared in posttreatment specimens without the same readout in a baseline specimen where available.

Recombinant Phenotyping

The drug susceptibility phenotypes of presumptive resistance mutations were determined by recombinant transfer of the

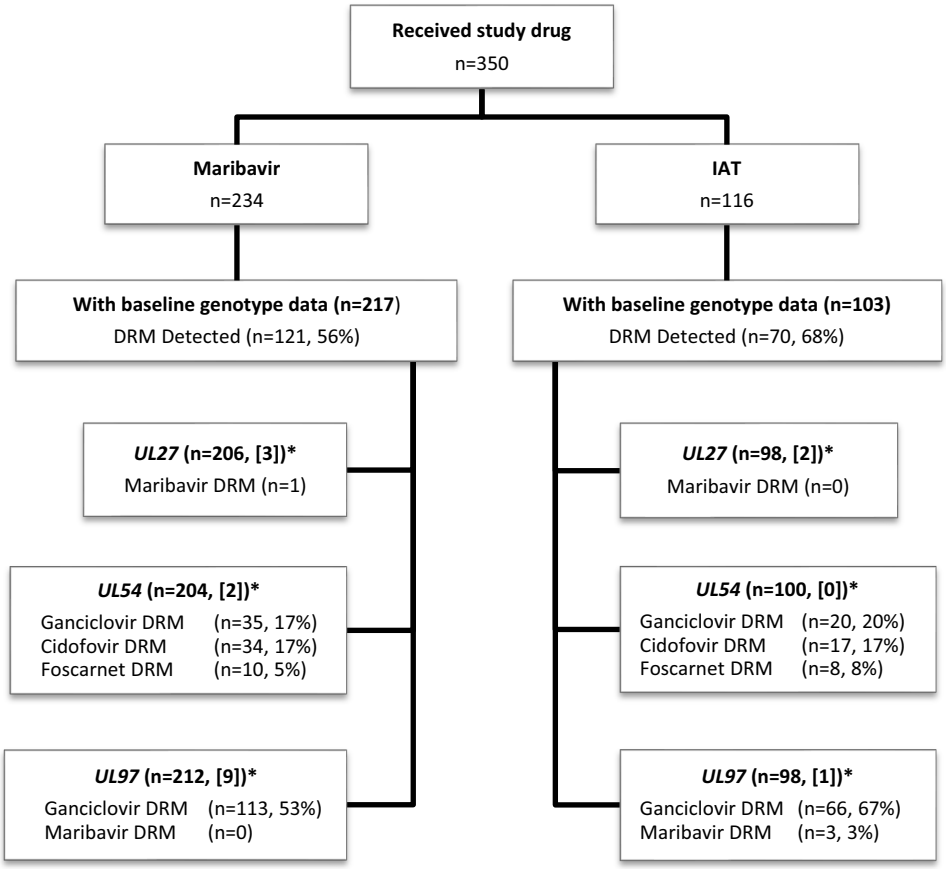

Figure 1. Baseline drug resistance mutations by assigned drug and viral gene. Asterisks indicate number of patients with genotyping in the indicated gene, and in square brackets the number of patients with incomplete data for the gene. Abbreviations: DRM, drug resistance mutation; IAT, investigator-assigned therapy.

individual mutations to a baseline bacterial artificial chromosome clone of CMV laboratory strain AD169 followed by a reporter-based yield reduction assay to determine the drug concentration required to reduce viral growth by 50% (EC_{50}), as previously published [10]. Resistance is defined as a drug EC_{50} > 1.9-fold increased over that of a wild-type control.

RESULTS

Sequence Variants Observed at Baseline

At baseline, genotyping for 1 or more viral genes (*UL27*, *UL54,* and *UL97*) was available for 320 (91%) of the 350 individuals in the resistance analysis group. Those without data in any gene (n = 30) had baseline viral loads of less than 455 IU/mL, too low for reliable genotyping. Figure 1 shows the frequency of detection of baseline DRMs according to the randomized treatment and viral gene. As expected, *UL97* ganciclovir-DRMs were the most common, detected in 53% and 67%, respectively, of those assigned to maribavir and IAT who had baseline *UL97* data. Considering both *UL97* and *UL54* baseline data, 121 (56%) patients receiving maribavir and 70 (68%) receiving IAT had genotypic evidence of IAT-DRMs. Baseline resistance to maribavir was limited to 1 instance of *UL27* L193F (maribavir arm) and 3 instances of *UL97* F342Y (IAT arm).

Supplementary Table 1 lists the specific DRMs detected at baseline. The 26 distinct *UL97* amino acid substitutions confirm the historical status of M460V/I, H520Q, C592G, A594V, L595S, and C603W as the 7 most common ganciclovir resistance mutations [8], although the low-grade resistance substitution C592G appears to be declining in frequency [14]. Adding to the 7 canonical mutations are others in the established codon range 590–607, including in-frame codon deletions, as well as the more recently identified K359N/Q and F342Y [15]. The 33 distinct *UL54* mutations include the exonuclease domain mutations mapping to codons 408–545 that characteristically confer dual cidofovir-ganciclovir resistance, followed by amino-terminal, palm and finger domain mutations conferring primarily foscarnet resistance, with notable triple-drug resistance resulting from Q578H, A834P, G841A, and D981del2 [8]. The *UL27* substitution L193F was observed in vitro [16] to confer low-grade (2.6-fold increased EC_{50}) maribavir resistance.

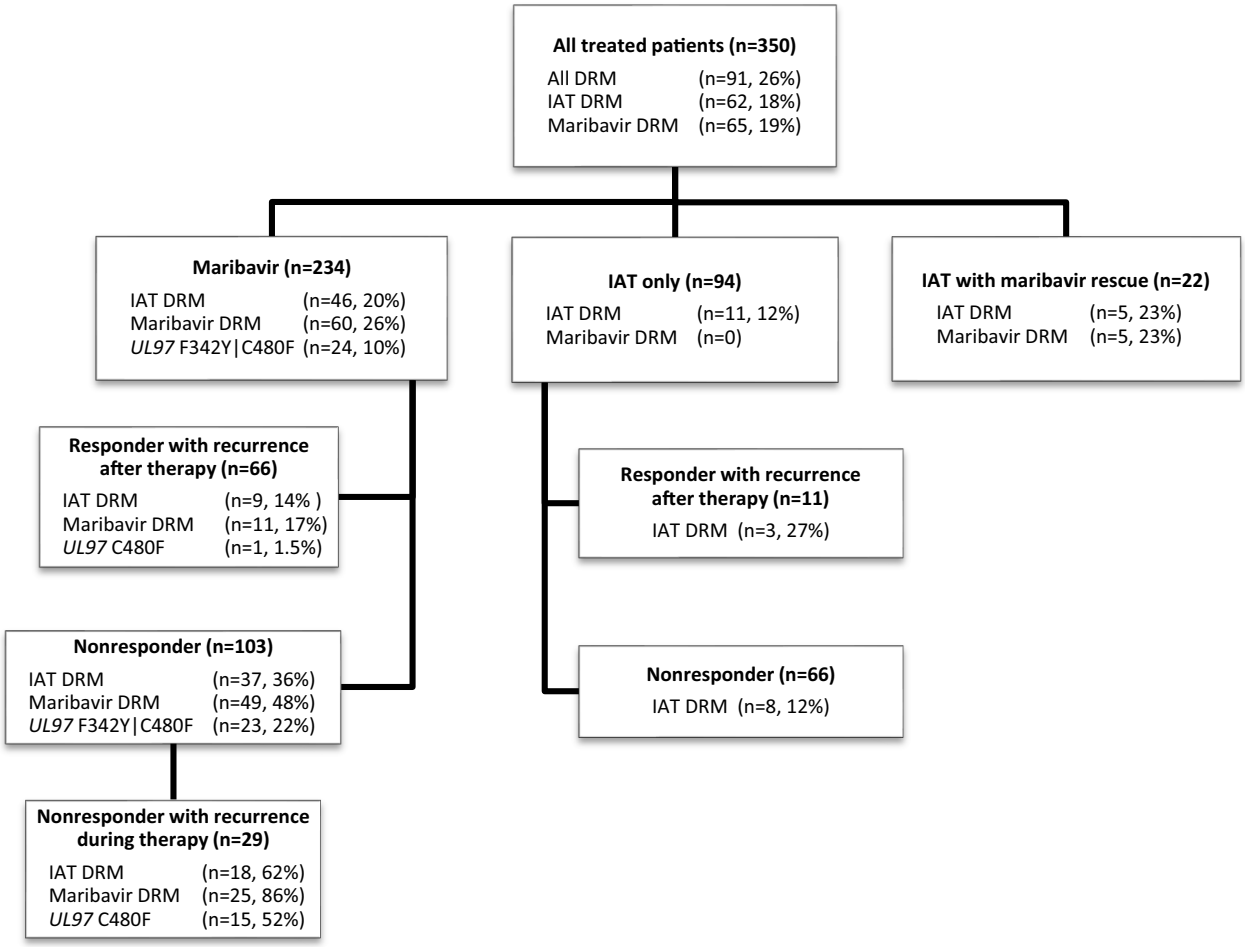

Figure 2. Treatment-emergent drug resistance mutations by patient category. Each treatment and outcome category is listed in bold with the number of patients (n). The number and percent of each group that developed maribavir-DRMs or IAT-DRMs is listed. *UL97* F342Y and C480F are counted under both maribavir-DRMs and IAT-DRMs, and also listed separately if present. Abbreviations: DRM, drug resistance mutation; IAT, investigator-assigned therapy.

Supplementary Table 2 lists 175 uncharacterized amino acid variants encountered at baseline that are not classified as known polymorphisms or resistance mutations. As in prior CMV drug trials [17], the list is long, especially for *UL54*.

Baseline Drug Resistance Mutations and Subsequent Treatment Response

Among 121 maribavir recipients with detected baseline IAT-DRMs (Figure 1), 76 (63%) had a treatment response, as compared with the 56% of all study patients who received maribavir [3]. The 76 responders received a median of 57 days of maribavir treatment (range 54–60 days). The patient with baseline *UL27* L193F denoting low-grade maribavir resistance (Figure 1) was a nonresponder; the mutation persisted over the 8-week maribavir treatment duration and *UL97* F342Y additionally evolved beginning at 4 weeks. Among those randomized to IAT (Figure 1), the 70 with any detected baseline IAT-DRMs included 15 (21%) treatment responders, as compared with a 30% response rate in the 33 IAT recipients without a detected baseline DRM. Premature discontinuations strongly affected response rates to IAT [3]. All 15 responders received ≥28 days of study drug (median 56 days, range 28–58). Of the 55 nonresponders, only 32 (58%) received ≥28 days of study drug (median 36, range 4–64). Among 50 patients receiving foscarnet, only 1 (a nonresponder treated for 19 days) had a baseline foscarnet-DRM. The foscarnet response rate of 26% rose to 10 (71%) of the 14 who received >52 days of therapy. The 47 patients receiving valganciclovir or ganciclovir (without foscarnet) had a 26% response rate. Of the 31 with a baseline ganciclovir-DRM, 5 (16%) were responders, as were 4 (27%) of 15 who received >52 days of therapy. None of 6 cidofovir recipients were responders despite a baseline cidofovir DRM detected in only 1. The 3 patients with *UL97* F342Y implying dual ganciclovir and maribavir resistance at baseline were all IAT nonresponders; 1 received ganciclovir and 2 received foscarnet.

Table 1. Distribution of Treatment-Emergent Maribavir DRM

rMed	Gene	Amino Acid Substitution	n	Fold Increase Maribavir EC_{50}	Reference for EC_{50}
IAT[a]	*UL97*	T409M	4	78–90	[10, 15]
IAT[a]	*UL97*	H411L	1	69	[18]
IAT[a]	*UL97*	H411Y	2	12–18	[15, 18]
Maribavir	*UL97*	F342Y[b]	3	4.5	[15]
Maribavir	*UL97*	T409M	30	78–90	[10, 15]
Maribavir	*UL97*	H411L	1	69	[18]
Maribavir	*UL97*	H411N	3	9	[18]
Maribavir	*UL97*	H411Y	24	12–18	[15, 18]
Maribavir	*UL97*	C480F[c]	21	224	[10]

Abbreviations: EC_{50}, drug concentration that reduces viral growth by 50%; DRM, drug resistance mutation; IAT, investigator-assigned therapy; rMed, randomized medication assignment.

[a]Genotyped after receiving maribavir rescue.

[b]Also has 6-fold increased ganciclovir EC_{50}.

[c]Also has 2.3-fold increased ganciclovir EC_{50}.

Sequence Variants Observed After Receiving Study Drug

The incidence of treatment-emergent (not detected at baseline) DRM in various study subsets is shown in Figure 2. Among 234 patients randomized to maribavir, posttreatment maribavir-DRMs were detected in 26%, and IAT-DRMs in 20%, including the 10% who developed mutations *UL97* F342Y and C480F classified as both maribavir-DRMs and IAT-DRMs. Among 94 patients randomized to IAT who did not receive maribavir rescue therapy, none developed maribavir-DRMs and 12% had IAT-DRMs detected. Among the 22 randomized to IAT and later switched to maribavir rescue therapy, all 5 who developed maribavir-DRMs did so after maribavir, while 3 of 5 who developed IAT-DRMs did so before maribavir treatment.

The incidence of maribavir resistance mutations varied by treatment outcome (Figure 2). Those who responded to maribavir but developed a CMV recurrence after the end of therapy had a 17% incidence of maribavir-DRMs, while those who did not respond had a 48% incidence of maribavir-DRMs, including 86% of nonresponders who had a rebound in viral load while on therapy after initially achieving a viral load below the quantitation limit.

Too few of those randomized to IAT developed treatment-emergent DRMs to allow a meaningful breakdown of incidence by the specific IAT drug and outcome; of the 16 (IAT with or without maribavir rescue) who developed IAT-DRMs (Figure 2), 7 received foscarnet, 5 received ganciclovir, and ≤2 received valganciclovir with or without foscarnet or cidofovir.

The relative frequency of treatment-emergent maribavir-DRMs is shown in Table 1. All emergent maribavir resistance was attributable to a total of only 6 *UL97* mutations. Amino acid substitutions T409M, H411Y, and C480F were by far the most common, and confer moderate to high-grade maribavir resistance. Combinations of maribavir-DRMs were observed in some patients, including 9 instances of H411Y and T409M, 6 instances of T409M and C480F, and 3 instances of H411Y and C480F, suggesting that the initial selection of a lower-grade DRM may lead to additional mutations that increase the level of maribavir resistance.

Among the 60 randomized to maribavir who developed maribavir resistance, the time from start of therapy to first detection of maribavir-DRMs ranged from 26 to 130 days (median 56 days); their starting viral loads varied widely from 145 to 1.67×10^6 IU/mL (median 10 277 IU/mL), as compared with a median of 3521 IU/mL for all who were randomized to maribavir. The distribution of starting viral loads and time to emergence of a maribavir resistance mutation is shown in Supplementary Figure 1, with poor correlation of these parameters (Pearson coefficient −0.17).

Table 2 lists the treatment-emergent IAT-DRMs in those who received maribavir or IAT. The maribavir-ganciclovir dual-resistant mutations *UL97* F342Y and C480F are omitted from this table because they are included in Table 1; no IAT recipient developed these mutations. A similar number of IAT and maribavir recipients developed a comparable number of IAT-DRMs that have no known connection with maribavir resistance, including *UL97* mutations that have been shown to confer no maribavir resistance. This finding makes it difficult to distinguish IAT-DRMs selected by prior IAT drugs (not detected at baseline) from mutations that were newly selected during the period of study drug exposure to maribavir or IAT.

Supplementary Table 3 lists the 64 uncharacterized treatment-emergent sequence variants detected in this trial. These are not documented polymorphisms, but not many are in the proximity of known DRMs. Almost all were detected in a single individual per variant. None of these variants plausibly alter the highly concentrated mapping of maribavir-DRMs to the mutations listed in Table 1.

Retreatment After Development of Maribavir Resistance

No protocol was specified for selecting and monitoring the treatment of the 65 who developed emergent maribavir-DRMs (Figure 2). Study records indicate that within 4 weeks of ending maribavir (or first appearance of maribavir-DRMs, if later), 31 received ganciclovir or valganciclovir, 27 received foscarnet, 2 received cidofovir, and 7 received letermovir. Some received more than 1 drug, and no treatment data were available for 13. Among the 52 with follow-up treatment history, 30 (58%) were recorded as having achieved viral clearance within 2 months of ending maribavir treatment, including 13 (62%) of 21 who developed *UL97* F342Y or C480F that confer ganciclovir cross-resistance. Among 19 who developed C480F, the 12 who were successfully treated included 8 who received only ganciclovir or valganciclovir. Foscarnet was used to treat 2 patients who developed F342Y, 1 successfully.

Phenotypes of Presumptive Resistance Mutations

Table 3 shows the drug susceptibility data of recombinant viruses validating the phenotypes of presumptive resistance mutations. The EC_{50} data confirm that all 9 newly constructed mutant viruses show some degree of drug resistance. The *UL97* mutants E596Q and A594P confer, respectively, lower and higher levels of ganciclovir resistance consistent with some other mutations at those codons. The various *UL54* exonuclease domain mutants N408H, L501F, T503A, K513Q, P522T, and L545F confer the characteristic ganciclovir and cidofovir dual resistance to varying degrees (borderline for P522T and higher level for K513Q/L545F). The *UL54* amino-terminal mutation C590F confers low-grade foscarnet resistance and a borderline <2-fold elevation of cidofovir and ganciclovir EC_{50}, comparable to findings reported for nearby mutations *UL54* S585A [19], D588E/N [20, 21], and F595I [19].

Table 2. Distribution of Treatment-Emergent IAT DRM

rMed IAT			rMed MBV		
Gene	Amino Acid Substitution	n	Gene	Amino Acid Substitution	n
UL54	F412V	1	*UL54*	S290R	2
UL54	L516P	2	*UL54*	N408D	1
UL54	P522S	1	*UL54*	L501I	1
UL54	L545S	1	*UL54*	T503I	1
UL54	Q578H	3	*UL54*	K513N	1
UL54	C590F	1	*UL54*	L516P	1
UL54	E756K	1	*UL54*	P522T	1
UL54	L773V	1	*UL54*	C590F	1
UL54	L776M	1	*UL54*	V715M	1
UL54	A809V	1	*UL54*	L773V	1
UL54	A834P	1	*UL54*	G841A	1
UL54	G841A	2	*UL54*	G841S	1
UL97	K359E	1	*UL54*	A987G	1
UL97	M460V	1	*UL97*	K359E	1
UL97	H520Q	1	*UL97*	M460V	2
UL97	A594V	2	*UL97*	H520Q	1
UL97	L595F	1	*UL97*	A594V	3
UL97	L595S	1	*UL97*	L595F	1
UL97	C603W	2	*UL97*	L595S	1
			UL97	E596G	1
			UL97	C603W	1

UL97 F342Y and C480F, listed in Table 1, are not repeated here.

Abbreviations: DRM, drug resistance mutation; IAT, investigator-assigned therapy; MBV, maribavir; rMed, randomized medication assignment.

DISCUSSION

In this phase 3 study, 26% of transplant recipients receiving up to 8 weeks of maribavir therapy for CMV infection after failing conventional therapy developed genotypic evidence of maribavir resistance. Treatment nonresponders, particularly those who experienced a viral load rebound while on therapy, were at higher risk and merit closer monitoring for resistance. The most common genotypic resistance markers for maribavir resistance are confirmed to be *UL97* T409M, H411Y, and C480F. Baseline resistance to standard therapy (IAT) did not preclude a treatment response to maribavir, and a majority of those who developed maribavir resistance eventually achieved viremia clearance with alternative therapy.

This resistance analysis is broadly in agreement with findings reported for the phase 2 maribavir treatment trials [10], now with a more complete genotypic dataset and comparison of

Table 3. Genotypes and Phenotypes of Recombinant Viruses

		Cidofovir EC_{50}, μM				Ganciclovir EC_{50}, μM				Foscarnet EC_{50}, μM			
Strain[a]	Genotype[b]	Mean	SD	n	Ratio[c]	Mean	SD	n	Ratio[c]	Mean	SD	n	Ratio[c]
Control strains													
4198	*UL54* WT	0.24	0.05	33	…	1.22	0.31	30	…	37	9	35	…
4200	*UL97* WT	…	…	…	…	1.31	0.25	9	…	…	…	…	…
4376	*UL54* D981del2	1.00	0.25	43	**4.2**	8.12	2.59	36	**6.7**	112	25	37	**3.0**
New recombinants (n = 9)													
4540	*UL54* N408H	0.79	0.21	12	**3.3**	2.38	0.41	10	**2.0**	41	8	10	1.1
4539	*UL54* L501F	0.91	0.20	10	**3.8**	3.12	0.72	8	**2.6**	48	11	10	1.3
4543	*UL54* T503A	0.88	0.25	15	**3.7**	3.79	1.24	10	**3.1**	55	11	14	1.5
4544	*UL54* K513Q	4.38	1.14	13	**18**	8.04	2.93	11	**6.6**	38	9	10	1.0
4541	*UL54* P522T	0.47	0.13	16	**2.0**	2.56	0.84	13	**2.1**	51	13	17	1.4
4542	*UL54* L545F	2.57	0.59	10	**11**	4.84	1.12	11	**4.0**	44	10	10	1.2
4499	*UL54* C590F	0.39	0.07	14	1.6	1.98	0.36	14	1.6	85	12	12	**2.3**
4538	*UL97* A594P	…	…	…	…	10.7	3.08	9	**8.1**	…	…	…	…
4537	*UL97* E596Q	…	…	…	…	3.61	0.83	8	**2.7**	…	…	…	…

Bold indicates EC_{50} > 1.9× WT control. All EC_{50} ratios >1.4 denote a significant EC_{50} shift from WT. $P < 1.1 \times 10^{-4}$, Student *t* test, 2-tailed unequal variances.

Abbreviations: EC_{50}, drug concentration that reduces viral growth by 50%; n, number of replicate assays; SD, standard deviation of the EC_{50} values; WT, wild type.

[a]Serial number of recombinant CMV strain.

[b]*UL54* amino acid substitution shown. D981del2 = in-frame deletion of codons 981–982.

[c]Ratio = EC_{50} of mutant virus/EC_{50} of WT control.

findings with IAT. Early in vitro studies had already established *UL97* T409M and H411Y as important maribavir-DRMs [18], but F342Y and C480F are more recently confirmed maribavir-DRMs [10, 15] that call for expanded diagnostic *UL97* genotyping beyond what was customary for assessing ganciclovir resistance [14]. These 4 mutations confer widely differing degrees of maribavir resistance (Table 1). The more common ones, T409M, H411Y, and C480F, likely abrogate any useful therapeutic activity of maribavir and may appear in combination to further degrade its effect. Despite the high-grade maribavir resistance conferred by C480F, the mutant is only moderately attenuated in growth in vitro [10, 22], consistent with its frequent emergence in treated patients (Table 1). This contrasts with *UL97* knockout strains of CMV that are highly resistant to maribavir but also severely growth impaired [5, 8, 10] and have not been authenticated in clinical specimens.

DRMs conferring dual resistance to maribavir and ganciclovir in clinical practice have gained more attention with the characterization of *UL97* F342Y [15] and C480F [10, 22]. Data from this trial showing detection of F342Y at baseline in 3 patients, as well as emerging after maribavir in 3 other patients, indicate that either maribavir or ganciclovir therapy can select for this mutation to confer cross-resistance to the other. On the other hand, C480F has not been reported as selected by prior ganciclovir therapy. The low-grade ganciclovir resistance conferred by C480F is comparable to that of the canonical *UL97* C592G substitution that has better growth fitness [10] and would not be expected to out-compete in the presence of ganciclovir. It remains possible that small subpopulations of C480F are selected after prior ganciclovir exposure to facilitate emergence after maribavir treatment. The successful treatment with ganciclovir or valganciclovir of 8 patients who developed C480F after maribavir is consistent with current management guidelines [23] that do not rule out use of ganciclovir in the presence of mutations conferring low-grade resistance. Apart from F342Y and C480F, the common maribavir and ganciclovir DRMs have not been reported to confer cross-resistance; instead, the ganciclovir-DRM M460V/I are hypersensitive to maribavir [14].

Remarkably, the first clinical detection of *UL27* mutation (substitution L193F) conferring maribavir resistance was at baseline. The origin of this mutation is unclear, as it has not been documented as a polymorphism [24], but maribavir resistance based on *UL27* mutation appears to be uncommon in clinical practice [10]. Few *UL27* variants emerged after exposure to maribavir (Supplementary Table 3), a comparable number emerged after IAT, and the variant codon loci are close to many documented *UL27* polymorphisms.

The documentation of additional IAT-DRMs (Table 3) extends the history of occasional appearance of novel DRMs even after decades of use of these drugs, although the ones validated here are mostly alternative amino acid substitutions at the same codons as known DRMs. Most of the uncharacterized variants listed in Supplementary Tables 2 and 3 are likely polymorphisms unrelated to resistance, but additional phenotypic analyses could reveal some new genotypic markers for resistance and risk of treatment failure.

Among limitations of the current study, this phase 3 trial offered no reliable comparison of the incidence of drug resistance after treatment with maribavir or IAT. Although 48% of maribavir nonresponders developed maribavir resistance mutations and 12% of IAT nonresponders developed IAT resistance mutations (Figure 2), interpretation is complicated by the premature discontinuation of IAT in many patients (shorter exposure to drug while on study) and selection of DRM by prior IAT drugs that may not have been detected at baseline. Posttreatment IAT resistance mutations (not detected at baseline) without maribavir cross-resistance appeared in similar proportions in both maribavir and IAT recipients (Figure 2), suggesting a fluctuating sensitivity of detection of mutations selected by prior antiviral therapy rather than the study drug. Validated deep sequencing technologies may offer improved sensitivity of detection of mutant subpopulations at baseline and during therapy [8]. Randomized studies that do not involve as many prior antiviral treatments or baseline DRMs, and adjusted for starting viral loads and duration of drug exposure, will be more informative as to the relative incidence of drug resistance after therapy with maribavir or other CMV antivirals.

In conclusion, viral mutations may appear after several weeks of maribavir treatment and their detection requires a wider scope of *UL97* genotypic testing than was prevalent in the past. This testing may be especially indicated where a rebound in viral load occurs while on maribavir therapy. Maribavir resistance mutations are generally distinct from those associated with other antivirals, except that certain *UL97* substitutions, such as F342Y or C480F, confer a degree of maribavir-ganciclovir cross-resistance that needs to be considered when selecting alternative therapy. Future research includes surveillance for other *UL97* or *UL27* mutations that may affect maribavir susceptibility.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. All authors were involved in analysis and interpretation of data, drafting the article and/or revising it critically for important intellectual content, and all authors

approved the final version to be published. S. C., M. F., R. A. M., and J. J. W. contributed analysis conception and design, and performed acquisition of data.

Acknowledgments. Justin Watanabe provided technical assistance with recombinant phenotyping. Lipika Ghosh, PhD, and Ginger Pocock, PhD, from Takeda Development Center Americas, Inc., Cambridge, MA, contributed to the data analysis and verification. Under the direction of the authors, Milda Jakutaviciute, PhD, employee of Excel Medical Affairs (Fairfield, CT), provided assistance in manuscript formatting, proofreading, and document assembly, but not in the composition of its content. Takeda Development Center Americas, Inc., Lexington, MA, provided funding to Excel Medical Affairs for this assistance.

Data sharing. The clinical trials datasets, including redacted study protocol, redacted statistical analysis plan, and individual participants' data behind the results reported in this article, will be available within 3 months from initial request, to researchers who provide a methodologically sound proposal. The data will be provided after its deidentification, in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization.

Financial support. This work was supported by Takeda Development Center Americas, Inc., Lexington, MA; National Institutes of Health (grant number R01-AI116635 to S. C.); and use of US Department of Veterans Affairs facilities and resources. Funding to pay the Open Access publication charges for this article was provided by Takeda Development Center Americas, Inc., Lexington, MA.

Potential conflicts of interest. J. J. W., R. A. M., and M. F. are Takeda employees and stockholders. N. E. B. is a paid consultant for Takeda. S. A. reports research grants or contracts paid to institution from BioMérieux, Biotest, Elitech, Hologic, Merck, Merck Sharp & Dohme, and Takeda; honoraria for lectures paid to institution from Biotest, Merck Sharp & Dohme France, and Takeda; support for attending meetings from Biotest, Merck Sharp & Dohme France; advisory board paid to institution from Biotest, Merck Sharp & Dohme, and Takeda; and primary investigator for Solstice 303 study in France. C. C. reports consulting fees from Merck and Takeda; honoraria for lectures from Avir Pharma and Takeda; and advisory board membership for Takeda. R. F. C. reports consultancy fees from Merck/MSD, Oxford Immunotec, Takeda, and Viracor-Eurofins. C. N. K. reports paid consultant/clinical trial adjudication, honoraria for lectures, meeting support, and expert testimony for Takeda. J. L. reports research grants from the Danish Government, Danish National Research Foundation, and National Institutes of Allergy and Infectious Diseases. G. A. P. has received partial funding from P30 CA008748 Cancer Center Support Grant; and consulting fees from AlloVir, Cidara, Octapharma, Takeda, MSK, Symbio, and Vera Armatta; research funding paid to the institution from MSD and Takeda; and honoraria from MSD. M. R. P. reports consulting fees from Union Therapeutics; honoraria for presentations from Takeda; and advisory board membership for Takeda. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Maertens J, Cordonnier C, Jaksch P, et al. Maribavir for preemptive treatment of cytomegalovirus reactivation. N Engl J Med **2019**; 381:1136–47.
2. Papanicolaou GA, Silveira FP, Langston AA, et al. Maribavir for refractory or resistant cytomegalovirus infections in hematopoietic-cell or solid-organ transplant recipients: a randomized, dose-ranging, double-blind, phase 2 study. Clin Infect Dis **2019**; 68:1255–64.
3. Avery RK, Alain S, Alexander BD, et al. Maribavir for refractory cytomegalovirus infections with or without resistance post-transplant: results from a phase 3 randomized clinical trial. Clin Infect Dis **2022**; 75:690–701.
4. Biron KK, Harvey RJ, Chamberlain SC, et al. Potent and selective inhibition of human cytomegalovirus replication by 1263W94, a benzimidazole L-riboside with a unique mode of action. Antimicrob Agents Chemother **2002**; 46: 2365–72.
5. Prichard MN. Function of human cytomegalovirus UL97 kinase in viral infection and its inhibition by maribavir. Rev Med Virol **2009**; 19:215–29.
6. Steingruber M, Marschall M. The cytomegalovirus protein kinase pUL97: host interactions, regulatory mechanisms and antiviral drug targeting. Microorganisms **2020**; 8:515.
7. Sullivan V, Talarico CL, Stanat SC, Davis M, Coen DM, Biron KK. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature **1992**; 358:162–4.
8. Chou S. Advances in the genotypic diagnosis of cytomegalovirus antiviral drug resistance. Antiviral Res **2020**; 176: 104711.
9. Chou S. Cytomegalovirus UL97 mutations in the era of ganciclovir and maribavir. Rev Med Virol **2008**; 18:233–46.
10. Chou S, Song K, Wu J, Bo T, Crumpacker C. Drug resistance mutations and associated phenotypes detected in clinical trials of maribavir for treatment of cytomegalovirus infection. J Infect Dis **2022**; 226:576–84.
11. Chemaly RF, Ullmann AJ, Stoelben S, et al. Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. N Engl J Med **2014**; 370:1781–9.
12. Chou S. Rapid in vitro evolution of human cytomegalovirus UL56 mutations that confer letermovir resistance. Antimicrob Agents Chemother **2015**; 59:6588–93.

13. Goldner T, Hempel C, Ruebsamen-Schaeff H, Zimmermann H, Lischka P. Geno- and phenotypic characterization of human cytomegalovirus mutants selected in vitro after letermovir (AIC246) exposure. Antimicrob Agents Chemother **2014**; 58:610–3.
14. Chou S, Watters M, Sinha R, Kleiboeker S. Ganciclovir and maribavir susceptibility phenotypes of cytomegalovirus UL97 ATP binding region mutations detected by expanded genotypic testing. Antiviral Res **2021**; 193:105139.
15. Chou S, Wu J, Song K, Bo T. Novel UL97 drug resistance mutations identified at baseline in a clinical trial of maribavir for resistant or refractory cytomegalovirus infection. Antiviral Res **2019**; 172:104616.
16. Chou S. Diverse cytomegalovirus UL27 mutations adapt to loss of viral UL97 kinase activity under maribavir. Antimicrob Agents Chemother **2009**; 53:81–5.
17. Chou S, Boivin G, Ives J, Elston R. Phenotypic evaluation of previously uncharacterized cytomegalovirus DNA polymerase sequence variants detected in a valganciclovir treatment trial. J Infect Dis **2014**; 209:1219–26.
18. Chou S, Marousek GI. Accelerated evolution of maribavir resistance in a cytomegalovirus exonuclease domain II mutant. J Virol **2008**; 82:246–53.
19. Gilbert C, Azzi A, Goyette N, Lin SX, Boivin G. Recombinant phenotyping of cytomegalovirus UL54 mutations that emerged during cell passages in the presence of either ganciclovir or foscarnet. Antimicrob Agents Chemother **2011**; 55:4019–27.
20. Chou S. Phenotypic diversity of cytomegalovirus DNA polymerase gene variants observed after antiviral therapy. J Clin Virol **2011**; 50:287–91.
21. Cihlar T, Fuller MD, Cherrington JM. Characterization of drug resistance-associated mutations in the human cytomegalovirus DNA polymerase gene by using recombinant mutant viruses generated from overlapping DNA fragments. J Virol **1998**; 72:5927–36.
22. Santos Bravo M, Plault N, Sanchez Palomino S, et al. Phenotype and genotype study of novel C480F maribavir-ganciclovir cross-resistance mutation detected in hematopoietic stem cell and solid organ transplant recipients. J Infect Dis **2021**; 224:1024–8.
23. Kotton CN, Kumar D, Caliendo AM, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation **2018**; 102:900–31.
24. Hantz S, Couvreux A, Champier G, et al. Conserved domains and structure prediction of human cytomegalovirus UL27 protein. Antivir Ther **2009**; 14:663–72.