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ORIGINAL RESEARCH

Cytomegalovirus Infection and the Implications of Drug-Resistant Mutations in Pediatric Allogeneic Hematopoietic Stem Cell Transplant Recipients: A Retrospective Study from a Tertiary Hospital in China

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ABSTRACT

Introduction: Drug-resistant cytomegalovirus (CMV) infection remains a challenge in the management of pediatric recipients of hematopoietic stem cell transplantation (HSCT). In this study, we retrospectively reviewed the clinical data on pediatric recipients of HSCT and identified known and unknown drug-resistant CMV variants.

Methods: A total of 221 children underwent allogeneic HSCT between October 2017 and November 2019 at Shenzhen Children's Hospital; of these, 35 patients were suspected of having drug-resistant CMV infections and were tested for drug-resistant mutations in the *UL97* and *UL54* genes by Sanger sequencing.

Results: Mutations in *UL97* or *UL54*, or in both, were detected in 11 patients. Most of these mutations have not been previously reported. The *UL97* mutation (A582V) was detected in only one patient who also harbored two *UL54* mutations (T760X and R876W). One patient with both the G604S and T691A mutations in the *UL54* gene died of CMV pneumonia. We investigated the risk factors associated with the development of drug-resistant CMV infection. Patients in whom both the donor and recipient had positive CMV serostatuses were less likely to have drug-resistant mutations (Fisher's exact test, $p < 0.05$).

Conclusion: Newly and previously detected CMV mutations in *UL97* and *UL54* may be associated with the development of drug-resistant CMV infection. The detection of these mutations may provide guidance for the management of post-transplant CMV infections.

Keywords: Cytomegalovirus; Hematopoietic stem cell transplantation; Pediatric; *UL54*; *UL97*

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Key Summary Points

Cytomegalovirus (CMV) infection is one of the most devastating complications and leading causes of pediatric recipients of allogeneic hematopoietic stem cell transplantation (HSCT).

Prolonged antiviral therapy may lead to the development of drug-resistance CMV variants.

This is one of the largest cohort studies investigating drug-resistant CMV mutations in pediatric HSCT recipients.

Some mutations identified in this study have been shown to have associations with antiviral drug susceptibilities or characterized as polymorphisms in previous literature. However, the roles of most of these mutations remain largely unknown and require further investigation.

The detection of drug-resistant CMV mutations may help to provide certain guidance for the management of post-transplant CMV infection in pediatric HSCT recipients.

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.14495763.

INTRODUCTION

Cytomegalovirus (CMV) infection remains one of the most devastating viral infections in recipients of hematopoietic stem cell transplantation (HSCT) [1]. Compared to adult patients, pediatric patients reportedly have higher risks of developing post-transplant CMV infections [2, 3]. Although end-organ diseases caused by CMV infection after HSCT are rare, CMV infections are associated with higher mortality, increased risks of developing complications at affected sites, and life-threatening infections with other pathogens [4, 5].

Currently, there are no standard guidelines for the prevention of CMV infection in pediatric recipients of HSCT. Antiviral drugs might be used as prophylactic, preemptive, or therapeutic strategies [2, 6]. However, the prolonged and repeated administration of antiviral drugs could cause resistance to these drugs, thereby resulting in treatment failure [7]. Therefore, the early identification of drug-resistant CMV variants is necessary to allow for appropriate therapeutic modifications in patients who do not have good response to standard treatment [8]. In this study, we retrospectively reviewed the clinical data on pediatric recipients of HSCT, and identified known and unknown drug-resistant CMV variants, focusing on mutations in the *UL97* and *UL54* genes, as well as their clinical relevance.

METHODS

Patients

A total of 221 patients who were younger than 18 years of age underwent allogeneic HSCT between October 2017 and November 2019 at the Shenzhen Children's Hospital, China. The follow-up period for at least 6 months following transplantation. Patient data, including clinical characteristics, treatments and outcomes, and laboratory test results, including donors' and recipients' CMV serostatuses, post-transplant plasma titers of CMV DNA, evidence of infections with other pathogens, and CMV genetic profiles were retrospectively reviewed. Written informed consents were obtained from parents or guardians for the collection, analyses, and publication of the patients' data. This study was approved by the Institutional Review Board of Shenzhen Children's Hospital (ethics approval number 202000302) and was conducted in

accordance with the Helsinki Declaration of 1964 and its later amendments.

CMV Infection Monitoring

The CMV serostatuses of donors and recipients were determined before HSCT. Plasma CMV DNA was monitored using real-time polymerase chain reaction (PCR) assay on a weekly basis for the first 3 months after HSCT, and at every outpatient visit thereafter. The CMV viral load was calibrated per the World Health Organization Standard for Human Cytomegalovirus for nucleic acid amplification techniques, NIBSC code 09/162 (1 IU/mL = 8 copies/mL) [9]. CMV DNAemia was defined as plasma levels of CMV DNA over 4×10^2 copies/mL (50 IU/mL). Refractory CMV DNAemia was defined as persistent CMV DNA that was elevated by more than 1 log10 or persistent viral load after 2 weeks, or less than 1 log10 decline, or less than 1 log10 elevation after 2 weeks of appropriately dosed antiviral therapy [10]. Recurrent CMV DNAemia was defined as CMV DNAemia at least 4 weeks after clearance of the previous infection. CMV diseases were diagnosed according to the clinical manifestations and the detection of CMV DNA at affected sites. Refractory CMV disease was defined as non-resolution of or lack of improvement in CMV-related symptoms after 2 weeks of appropriate antiviral therapy [7, 11]. Patients with CMV diseases, those with refractory CMV DNAemia, or those suspected of having drug-resistant CMV infections based on clinical evaluations were tested for drug-resistant mutations (DRMs) in *UL97* and *UL54*.

Detection of DRMs in *UL97* and *UL54*

The DRMs in *UL97* and *UL54* were detected using Sanger sequencing [12]. CMV DNA for sequencing analysis was extracted from whole blood using an Aidlab total nucleic acid extraction kit (Aidlab Biotechnologies, Beijing, PRC) according to the manufacturer's instructions. Purified nucleic acids were then amplified to create amplicons spanning codons 420–640 of *UL97* and codons 282–999 of *UL54* using an Applied Biosystems 7500 PCR amplifier (Applied Biosystems, Foster City, CA, USA). DNA was sequenced using an Applied Biosystems 3730XL DNA Analyzer (Applied Biosystems). Raw sequence data were analyzed using QIAGEN Variant Reporter software (version 1.1, QIAGEN, Redwood City, CA, USA).

Treatment for CMV Infection

Preemptive therapy was initiated in patients with plasma CMV DNA of at least 1×10^3 copies/mL (125 IU/mL) and in those with symptoms of CMV diseases, regardless of their CMV DNA level. Ganciclovir (GCV) was administered intravenously at 5 mg/kg every 12 h as first-line treatment. For patients suspected of having developed drug-resistant CMV infections, foscarnet (FOS) was administered intravenously at 60 mg/kg every 8 h with or without GCV. The course of antiviral treatment was guided by the monitoring of plasma CMV DNA titers, and the treatment continued until a negative plasma CMV DNA test result was obtained.

Statistics

Characteristics of patients with suspected drug-resistant CMV infections were analyzed and compared between those with and without DRMs. Categorical variables were compared using Fisher's exact test. Continuous variables were compared using the Mann–Whitney *t* test. A p value less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA).

RESULTS

Patient Cohort and Detection of *UL97* and *UL54* Mutations

A total of 221 pediatric patients received allogeneic HSCT between October 2017 and November 2019; of these, 112 had matched related or unrelated donors and 109 had

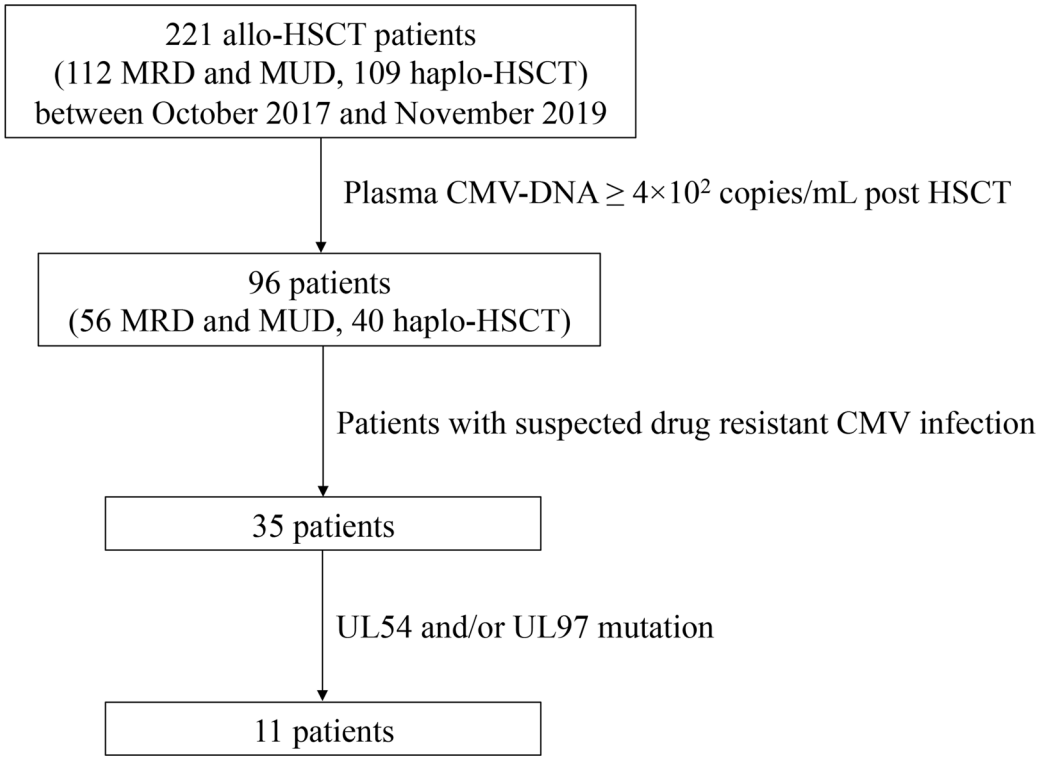

Fig. 1 Selection criteria of pediatric recipients of hematopoietic stem cell transplants that have been tested for mutations in the *UL97* and *UL54* genes by Sanger sequencing. Allo-HSCT allogeneic hematopoietic stem cell transplantation, MRD matched related donor, MUD matched unrelated donor, haplo-HSCT haploidentical hematopoietic stem cell transplantation

haploidentical donors. CMV DNAemia (plasma CMV DNA greater than 4×10^2 copies/mL, or 50 IU/mL) was observed in 96 (43%) patients after HSCT. Thirty-five (16%) patients were suspected of having developed drug-resistant CMV infections and were tested for DRMs in *UL97* and *UL54*.

In order to identify the drug-resistant CMV variants, CMV DNA was extracted from all 35 patients with suspected drug-resistant infections and tested for mutations in *UL97* and *UL54* via Sanger sequencing (Fig. 1). As shown in Table 1, 11 of 35 patients harbored mutations in *UL97* and *UL54*, either singly or in combination. Only one patient (patient 26) harbored a mutation (A582V) in *UL97*. This patient also harbored the T760X and R876W mutations in *UL54*. The other 10 patients harbored one or more mutations in *UL54*. The T691A mutation in *UL54* was detected in patients 2 and 29. Patient 29 also harbored the *UL54* G604S mutation and died as a result of CMV-related pneumonia. The A582V mutation in *UL97* and the G604S, G633F, G678S, and T691A mutations in *UL54* have been previously reported [12–18]. We additionally identified some novel *UL54* mutations, namely E353Q, D514N, A614S, G677D, M727I, T760X, T885M, R876W, G878E, V973A, and L999I.

Patient Characteristics

Clinical data of patients with suspected drug-resistant CMV infection is listed in Table 2. The age range was 1 to 14 years (median 7 ± 3 years), and most patients (71%) were female. Sixty-nine percent of patients had haploidentical donors, and 31% had matched related or unrelated donors. The CMV serostatuses were positive for both donors and recipients in most patients (66%), and this was associated

Table 1 Detection of mutations in *UL97* and *UL54* genes

UL54				*UL97*		
Patient no.	**Mutation**	**Reported in previous literature**	**Drug resistance**	**Mutation**	**Reported in previous literature**	**Drug resistance**
2	T691A	Yes [17, 18]	Polymorphism			
4	A614S	No	Uncertain			
5	G878E	No	Uncertain			
13	S633F	Yes [17, 18]	Polymorphism			
			Uncertain			
	V973A	No				
19[a]	L999I	No	Uncertain			
21	E353Q	No	Uncertain			
	D514N	No	Uncertain			
26	T760X	No	Uncertain	A582V	Yes [13, 14]	Polymorphism
	R876W	No	Uncertain			
29[b]	T691A	Yes [17, 18]	Polymorphism			
	G604S	Yes [12]	Uncertain			
32	M727I	No	Uncertain			
34	G678S	Yes [15, 16]	Resistance to GCV and CDV, sensitive to FOS			
	T885M	No	Uncertain			
35	G677D	No	Uncertain			

GCV ganciclovir, *CDV* cidofovir, *FOS* foscarnet

[a] This patient developed one relapse of CMV DNAemia

[b] Deceased patient

with lower risks of developing DRMs after transplantation (Fisher's exact test, $*p = 0.0223$). None of the patients had exposure to anti-CMV drugs before HSCT.

The primary diseases observed in the patients were thalassemia major (TM, 91%), severe aplastic anemia (SAA, 6%), and dyskeratosis congenita (DC, 3%). Patients with TM received myeloablative conditioning chemotherapy, and those with SAA and DC received reduced-intensity conditioning chemotherapy. Prophylactic treatment for graft versus host disease (GVHD) included anti-thymocyte globulin, cyclosporine, tacrolimus, or mycophenolate mofetil. Four patients received rituximab because of a high anti-donor-specific antigen before HSCT. One patient received methylprednisolone to treat autoimmune hemolytic anemia, and another patient with TM received splenectomy before transplantation.

Median time to myeloid engraftment was 20 ± 7 days (mean ± SD). Nine (26%) patients developed acute GVHD (aGVHD), and six (17%) patients developed grade II–IV aGVHD. Chronic GVHD (cGVHD) was observed in 4 (11%) patients. However, only one of them developed

Table 2 Characteristics of patients with suspected drug-resistant CMV infections

Patient characteristics	*n* (%), or mean ± SD			*p* value[a]
	Total patients	DRMs	No DRMs	
Number of patients	35	11 (31%)	24 (69%)	–
Female	20/35 (71%)	8/11 (73%)	12/24 (50%)	0.2814
Age at transplant	7 ± 3, range 1–14 years	7 ± 2 years	8 ± 3 years	0.6782
Primary disease				
Thalassemia major	32/35 (91%)	11/11 (100%)	21/24 (88%)	0.5361
Severe aplastic anemia	2/35 (6%)	0/11	2/24 (8%)	–
Dyskeratosis cogenita	1/35 (3%)	0/11	1/24 (4%)	–
Splenectomy[b]	1/35 (3%)	0/11	1/24 (4%)	> 0.9999
Graft type				0.4354
Matched donor	11/35 (31%)	2/11 (18%)	9/24 (38%)	–
Haploidentical donor	24/35 (69%)	9/11 (82%)	15/24 (62%)	–
Use of immunosuppressant before HSCT				
rATG	35/35 (100%)	11/11 (100%)	24/24 (100%)	–
Rituximab[c]	4/35 (11%)	2/11 (18%)	2/24 (8%)	0.5748
Steroid[d]	1/35 (3%)	0/11	1/24 (4%)	> 0.9999
aGVHD	9/35 (26%)	4/11 (36%)	5/24 (21%)	0.4159
Grade II–IV aGVHD	6/35 (17%)	2/11 (18%)	4/24 (17%)	> 0.9999
cGVHD	4/35 (11%)	2/11 (18%)	2/24 (8%)	0.5748
Severe cGVHD	1/35 (3%)	0/11	1/24	> 0.9999
Days to myeloid engraftment	20 ± 7 days	20 ± 5 days	20 ± 8 days	0.7537
Graft failure	0/35	0/11	0/24	–
CMV serostatus before HSCT				
D−/R−	4/35 (11%)	2/11 (18%)	2/24 (8%)	0.5748
D−/R+	1/35 (3%)	1/11 (9%)	0/24	0.3143
D+/R−	7/35 (20%)	4/11 (36%)	3/24 (13%)	0.1715
D+/R+	23/35 (66%)	4/11 (36%)	19/24 (79%)	0.0223*
Mismatched CMV serostatus	8/35 (23%)	5/11 (45%)	3/24 (13%)	0.0767
Positive urine CMV DNA before HSCT	10/35 (29%)	5/11 (45%)	5/24 (21%)	0.1344
Infection with other pathogens	18/35 (51%)	5/11 (45%)	13/24 (54%)	> 0.9999
EBV DNAemia	15/35 (43%)	4/11 (36%)	11/24 (46%)	0.4928
EBV-PTLD	1/35 (3%)	0/11	1/24 (4%)	> 0.9999
HSV-1	1/35 (3%)	0/11	1/24 (4%)	> 0.9999

Table 2 continued

Patient characteristics	*n* (%), or mean ± SD			*p* value[a]
	Total patients	DRMs	No DRMs	
B19V	1/35 (3%)	1/11 (9%)	0/24	0.3143
Tuberculosis	1/35 (3%)	0/11	1/24 (4%)	> 0.9999

DRM drug-resistant mutation, *HSCT* hematopoietic stem cell transplantation, *rATG* rabbit anti-thymocyte globulin, *aGVHD* acute graft versus host disease, *cGVHD* chronic graft versus host disease, *CMV* cytomegalovirus, *EBV* Epstein–Barr virus, *PTLD* post-transplant lymphoproliferative disease, *HSV-1* type 1 herpes simplex virus, *B19V* parvovirus B19

*$p < 0.05$

[a] Fisher's exact test for categorical variables, Mann–Whitney *U* test for continuous variables

[b] Splenectomy was performed in a patient with thalassemia major 2 years prior to HSCT

[c] Rituximab was given intravenously at 375/m^2 per week for 2–4 consecutive weeks to reduce the titer of donor-specific antibody before HSCT

[d] One patient received methylprednisolone intravenously and then orally for treatment of autoimmune hemolytic anemia before HSCT

severe cGVHD. Fifty-two percent of patients developed infection with other viruses. Epstein–Barr virus, type 1 herpes simplex virus, and parvovirus B19 infections were observed in 16 (46%), one (3%), and one (3%) patients, respectively. One (3%) patient developed pulmonary tuberculosis with CMV DNAemia (3%). Co-infections with other bacteria or fungus were not observed. No other significant differences were observed between patients with or without DRMs.

CMV Infection and Treatment

As shown in Table 3, the median interval from HSCT to the occurrence of CMV DNAemia was 42 ± 13 days (mean ± SD). The mean CMV DNA titer at the onset of DNAemia was 8.0 ± 1.33 × 10^3 copies/mL (mean ± SD) and increased by 10.83 ± 30.01-fold (mean ± SD) at the peak titer. Interestingly, patients without DRMs had higher-fold, but not significant, increases in viral titers compared to those with DRMs. Additionally, patients without DRMs had a higher risks of developing CMV-related diseases. However, this difference was also not significant. Five (14%) patients developed CMV diseases; four (11%) developed CMV-related pneumonia and one (3%) developed both CMV-related pneumonia and retinitis.

Among the patients who received antiviral medications, 51% received both GCV and FOS, and the remaining patients received either GCV (29%) or FOS (17%) alone. Notably, one patient received only intravenous immunoglobulin therapy without other antiviral medications. The patient tested negative for plasma CMV DNA at 18 days after the initial detection. Among the patients with refractory or persistent CMV diseases who did not show satisfactory responses to conventional treatment, three received transfusion of CMV-specific cytotoxic T lymphocytes (CTLs). These patients showed reductions of CMV viral loads below 500 copies/mL after two infusions of CTLs. No differences in treatment regimens were observed between patients with and without DRMs.

The durations of CMV DNAemia in patients with and without DRMs were 23 ± 13 and 29 ± 18 days, respectively. Five (14%) patients experienced CMV DNAemia more than once. One patients with DRMs developed severe CMV-related pneumonia and died during the initial CMV infection. Another patient without DRMs had right eye blindness caused by CMV retinitis. The remaining patients recovered well after appropriate treatment with antiviral therapy.

Table 3 Treatment and outcomes of patients with and without drug-resistant related mutations

	n (%), or mean ± SD			*p* value[a]
	Total patients	**DRMs**	**No DRMs**	
Days to CMV DNAemia post HSCT[b]	42 ± 13 days	39 ± 12 days	44 ± 14 days	0.46
CMV DNA titer at onset (copies/mL)	$8.0 \pm 13.3 \times 10^3$	$4.1 \pm 5.3 \times 10^3$	$9.8 \pm 15.4 \times 10^3$	0.99
CMV DNA titer at onset (IU/mL)	$1.0 \pm 1.7 \times 10^3$	$0.5 \pm 0.7 \times 10^3$	$1.2 \pm 1.9 \times 10^3$	0.99
Peak CMV DNA titer increase by fold	10.8 ± 30.1	2.0 ± 2.0	14.9 ± 35.8	0.24
CMV disease[c]	5/35 (14%)	1/11 (9%)	4/24 (17%)	0.99
CMV pneumonia	4/35 (11%)	1/11 (9%)	3/24 (13%)	0.99
CMV pneumonia + retinitis	1/35 (3%)	0	1/24 (4%)	–
Duration to initiate antiviral treatment	4 days[d]	4 days	3 days[d]	0.48
Treatment				
GCV	10/35 (29%)	2/11 (18%)	8/24 (33%)	0.43
FOS	6/35 (17%)	2/11 (18%)	4/24 (17%)	> 0.99
GCV + FOS	18/35 (51%)	7/11 (64%)	11/24 (46%)	0.47
No antiviral treatment	1/35 (3%)	0	1/24 (4%)	–
CTL	3/35 (8%)	1/11 (9%)	2/24 (8%)	> 0.99
Duration of CMV DNAemia[e]	28 ± 17 days	23 ± 13 days	29 ± 18 days	0.3845
Episodes of CMV DNAemia				
1 episode	30/35 (86%)	10/11 (91%)	20/24 (83%)	> 0.9999
2 episodes	4/35 (8%)	1/11 (9%)	3/24 (13%)	> 0.9999
3 episodes	1/35 (3%)	0/11	1/24 (4%)	–
Death[f]	1/35 (3%)	1/11 (9%)	0	–
Alive with complications[g]	1/35 (3%)	0	1/24 (4%)	–

DRM drug-resistant related mutation, *CMV* cytomegalovirus, *HSCT* hematopoietic stem cell transplantation, *GCV* ganciclovir, *FOS* foscarnet, *CTL* cytotoxic T lymphocyte

[a] Fisher's exact test for categorical variables, Mann–Whitney *t* test for continuous variables

[b] Four patients developed CMV DNAemia during prophylactic treatment with foscarnet

[c] All patients with confirmed CMV diseases had CMV pneumonia

[d] Excluding one patient that did not receive treatment with antiviral drugs

[e] Plasma CMV DNA was assessed by real-time polymerase chain reaction assay

[f] Patient died of severe respiratory failure caused by CMV pneumonia

[g] This patient had blindness of the right eye caused by CMV retinitis and chronic GVHD

DISCUSSION

In our study, or the 221 recipients of allogeneic HSCT, we identified 35 patients with suspected drug-resistant CMV infection. As prolonged and repeated antiviral treatment for CMV infections could result in the development of drug-resistant viral variants, patients with suspected drug-

resistant CMV infections should be screened for DRMs to allow for early interventions [7, 11]. In the current study, patients did not have anti-CMV treatment before HSCT. However, patients with positive CMV statuses suggested prior exposure to CMV before transplant. Post-transplant CMV status was monitored by screening plasma CMV DNA levels on a weekly basis for the first 3 months after HSCT, and then at every return visit thereafter. Patients were considered to have CMV DNAemia if their plasma CMV DNA levels were at least 400 copies/mL (50 IU/mL). However, preemptive antiviral therapies were administered only if the patients had rising plasma CMV DNA levels of at least 1000 copies/mL (125 IU/mL) or had developed symptoms suggestive of CMV diseases, regardless of viral load.

We examined DRMs by identifying mutations in *UL97* and *UL54* in patients with drug-resistant CMV infections. GCV-resistant mutations are observed more frequently in *UL97* [19, 20]. In clinical isolates, known CMV-resistant mutations are mainly located in the highly conserved region between codons 439 and 641 [21]. Less frequently, GCV resistance results from mutations in the DNA polymerase *UL54*. Mutations in *UL54* also confer resistance to most known DNA polymerase inhibitors, including GCV, valaciclovir (VACV), FOS, and cidofovir (CDV) [22, 23]. Other drug-resistant CMV variants, including those with mutations in the CMV DNA terminase complex members *UL56*, *UL89*, and *UL51*, are known to confer reduced susceptibility to antivirals such as letermovir (LTV) [19, 24–26]. However, mutations in *UL89* and *UL54* are only observed under in vitro selection pressure with drugs. Identification of these CMV variants in clinical isolates and their relevance to patient outcomes require further investigation.

GCV remains the first-line treatment for post-HSCT CMV infection. Some of the known mutations in *UL97* confer a high level of GCV resistance, and in such cases, the use of alternative drugs is recommended [27, 28]. However, it is difficult to determine the optimal antiviral therapy in patients with mutations that confer low-level GCV resistance or new mutations with unknown effects on drug sensitivity [19]. Inadequate dosing or differences in drug concentrations at infected sites could contribute to treatment failure [29]. In this study, patients received GCV at a full dose of 5 mg/kg every 12 h. Some studies recommended that recipients of solid organ transplants that show low-level GCV resistance may be treated with an escalation of GCV doses up to 10 mg/kg every 12 h, but this could increase the risks of renal toxicity [30]. Thus, close monitoring of the pharmacokinetics of antivirals and renal functions in patients is required if this therapeutic strategy is applied to pediatric recipients of HSCT in the future.

In this study, more than half of the patients with suspected drug-resistant CMV infections were administered FOS with GCV. Additionally, FOS monotherapy was administered in six patients who had risk factors associated with the development of drug-resistant CMV infection, such as receiving of a haploidentical graft, incompatible CMV serostatuses between the donor and the recipient, GVHD, and the intensity of immunosuppressive therapy. Therapy with FOS was not associated with a higher risks of developing DRMs. CDV is generally considered as a third-line treatment for post-HSCT CMV infections [7]. Recently, LTV has been well accepted as prophylaxis in patients with HSCT [31]. Other novel antiviral agents, including maribavir, brincidofovir, and leflunomide, are being evaluated for their safety and treatment efficacies for CMV infections in HSCT recipients [32–34]. However, none of these novel antiviral drugs is currently approved in China; hence, this limits the treatment options for patients in this study. In the current study, patients with refractory or resistant CMV diseases that did not respond to conventional treatment were transfused of CMV-specific CTLs and achieved good responses. Previous studies have demonstrated that CTLs may provide protective immunity for both early and late CMV infections, and these have been applied as first-line treatment combined with conventional antivirals against CMV infection in HSCT recipients in a recent clinical trial [35, 36].

Eleven (5%) patients harbored mutations in *UL97* and *UL54*. However, most of the DRMs detected in this study have not been previously

identified in clinical samples; hence, their effects on drug sensitivity remain uncertain. Only one patient (patient 26) harbored the *UL97* (A582V) mutation. This mutation has been previously reported in blood samples from solid organ transplant recipients. However, in vitro sensitivity analyses suggested that this CMV variant was sensitive to GCV [13, 14]. Additionally, this patient was found to harbor the T760X and R876W mutations in *UL54*. Whether the combination of mutations in these genes can decrease drug sensitivity remains unknown, because this patient achieved a favorable outcome and did not develop CMV-related diseases or relapse after antiviral treatment.

Ten patients harbored *UL54* mutations, including the E353Q, D514N, G557D, G604S, A614S, G678S, M727I, G878E, T885M, V973A, and L999I mutations. Of these, only the G604S, S633F, G678S, and T691A mutations have been reported previously [12, 15–17]. Our study is the first to report the other mutations, which should be added to the extensive list of known *UL54* mutations that confer resistance to multiple antivirals. The T691A mutation was found in two patients (patients 2 and 29). Together with the S633F mutation, these mutations have been characterized as polymorphisms in previous literature [17]. Patient 29 also harbored the G604S mutation in *UL54*. These two patients had distinct outcomes. Patient 2 responded well to antivirals, whereas patient 29 died of severe CMV-related pneumonia 44 days after the initiation of antiviral therapy. Our data suggest that this mutation may confer resistance to anti-CMV treatment. However, the exact role of the G604S mutation remains uncertain because this has been previously observed in clinical isolates from recipients of solid organ transplantation and characterized with unknown significance [12]. Combinations of mutations in the *UL54* gene are known to increase the level of drug resistance [37, 38]. Additionally, in vitro studies have suggested that different mutations in genetic loci with low-grade resistance to LTV may combine to cause high-grade resistance or promote other mutations having the same effects [25]. Although we suspect that combined G604S and T691A mutations in *UL54* may lead to a multiplier effect on the suppression of antiviral therapies, the exact correlation and impact on treatment outcomes require further investigation.

We compared the clinical characteristics and patient outcomes between patients with and without drug-resistant CMV mutations. Among patients with CMV diseases or those with more than one episode of CMV DNAemia, only two patients harbored drug-resistant CMV variants (patients 19 and 29) (Table 1 in the supplementary material). However, we could have underestimated the number of DRMs as a result of the sensitivity of Sanger sequencing. A mutation can only be detected with Sanger sequencing if it is present in at least 20–30% of the viral population [39]. Additionally, we only assessed mutations in codons 420–640 in *UL97* and codons 282–999 in *UL54*. Although rarely reported, the existence of CMV mutations outside these regions is hitherto unknown. Future studies using deep sequencing may help to identify unknown mutations affecting the susceptibility to antiviral therapies [40, 41]. Another limitation is that we were not able to examine the pharmacokinetics of antivirals used in this study; hence, antiviral dosing for patients with low levels of DRM resistance may not have been optimized. Additionally, we were unable to find a significant correlation between the presence of DRMs and the development of drug-resistant CMV infections because of the low incidence of CMV infection.

CONCLUSIONS

Our data suggest that DRMs in CMV may have an impact on the drug susceptibility and outcomes of pediatric recipients of HSCT. The detection of these mutations may provide guidance for management of post-transplant CMV infections. However, further analysis is required to confirm the exact clinical correlation between these mutations and disease outcomes.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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