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Letermovir in Refractory and Resistant CMV After Lung Transplantation: Therapeutic Potential and Emergence of Resistance

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ABSTRACT

Background: Cytomegalovirus (CMV) infection remains a major challenge in lung transplant (LTx) recipients, particularly in antiviral-resistant or refractory (R/R) cases. Letermovir, effective for prophylaxis, has been used off-label as salvage therapy for R/R CMV infection; however, data on treatment-emergent resistance in LTx recipients remain limited.

Methods: We retrospectively analyzed 61 consecutive LTx recipients treated with letermovir for R/R CMV infection between March 2018 and August 2024.

Results: Overall, 21 patients (35%) developed R/R CMV under therapy and were genotypically tested; in 19 (31.1%), UL56 mutations conferring letermovir resistance were detected after a median of 96 [51–395] days. In contrast, 40 patients (65.6%) showed a > 1 log decline in viral load within 14.5 [5–31] days and viral clearance after 34 [6–126] days. Patients who developed resistance had significantly higher viral loads at initiation (35.100 [13.900–228.000] vs. 7.805 [1.173–30.350] IU/mL; $p = 0.018$) and less frequent positive CMV-specific immune responses (7.1% vs. 38.7%; $p = 0.038$). Time to 1 log decline was shorter in patients without resistance (16 [5–31] vs. 34 [17–58] days; $p < 0.001$). Multivariable regression identified slow viral load decline (OR 0.721; $p < 0.001$) and higher baseline viral load (OR 1.110; $p = 0.044$) as independent risk factors.

Conclusion: Letermovir use for active R/R CMV infection was associated with treatment-emergent resistance in approximately one-third of patients, particularly in those with high baseline viral loads and delayed virological response. These findings support

Abbreviations: BMI, body mass index; CMV, cytomegalovirus; CMV-CMI, CMV-specific cell-mediated immunity; CMVIgG, i.v. CMV-immune globulins; CsA, ciclosporin A; EC50, 50% effective concentration; FK-506, tacrolimus; HSCT, hematopoietic stem cell transplant; i.v., intravenous; LET, letermovir; LTx, lung transplantation; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; PCR, polymerase chain reaction; R/R, resistant or refractory; SOT, solid organ transplantation.

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cautious use of letermovir for treatment, preferably in selected patients with low viral loads or limited alternative therapeutic options, with close virological monitoring and early resistance testing.

1 | Introduction

Cytomegalovirus (CMV) remains a major infectious complication after transplantation and continues to contribute substantially to morbidity and mortality among recipients of solid organ transplants (SOT) and allogeneic hematopoietic stem cell transplantation (HSCT) [1]. Despite advances in antiviral prophylaxis and treatment, CMV infection remains difficult to manage in immunocompromised patients, particularly once resistance or refractoriness to antiviral therapy develops [2].

For many years, the therapeutic management of refractory or resistant (R/R) CMV infection has relied primarily on high-dose (val)ganciclovir, foscarnet, and cidofovir. Although these agents are effective in selected patients, their clinical use is frequently limited by considerable toxicity, including myelosuppression, nephrotoxicity, and electrolyte disturbances [2]. Furthermore, prolonged antiviral exposure, profound immunosuppression, and insufficient CMV-specific immune control facilitate ongoing viral replication and promote the selection of resistance-associated mutations, predominantly within the UL97 and UL54 genes [3, 4].

The introduction of letermovir and maribavir has substantially expanded the therapeutic landscape for CMV infection. Maribavir is currently recommended as first-line treatment for R/R CMV infection; however, treatment failure, intolerance, clinically relevant drug interactions, and the emergence of UL97 resistance mutations may restrict its long-term effectiveness in a subset of patients [2]. Consequently, additional antiviral strategies remain clinically relevant. Letermovir, a selective inhibitor of the CMV terminase complex, is licensed for CMV prophylaxis following allogeneic HSCT and in high-risk kidney transplant recipients (D+/R−) [5, 6]. Because its mechanism of action differs fundamentally from that of DNA polymerase inhibitors, cross-resistance with established anti-CMV drugs is uncommon, and the drug is generally well tolerated [7].

Beyond its approved prophylactic indications, letermovir has increasingly been used off-label for the treatment of R/R CMV infection. Several observational studies and case series have reported virological outcomes, particularly in patients with lower baseline viral loads or when administered as secondary prophylaxis [8–11]. Nevertheless, prospective evidence supporting its therapeutic use remains scarce, and current international consensus guidelines conclude that controlled clinical data are still insufficient to recommend letermovir as standard treatment for active CMV infection [2].

An important limitation of letermovir is its relatively low genetic barrier to resistance. Experimental studies have demonstrated rapid selection of resistance-associated UL56 variants under antiviral pressure, and treatment-emergent resistance has subsequently been documented in clinical practice [9, 12–14]. As off-label therapeutic use becomes more widespread, early identification of virological failure and resistance is increasingly important to optimize patient management [10].

Despite the growing experience with letermovir, the frequency, timing, and determinants of resistance development remain incompletely characterized, particularly in lung transplant recipients, who represent one of the transplant populations at highest risk for CMV-related complications [10, 15]. Improved knowledge of factors associated with resistance may facilitate patient selection, refine virological monitoring, and guide individualized antiviral treatment strategies. Therefore, the objective of the present study was to identify clinical predictors associated with the emergence of letermovir resistance in lung transplant recipients treated for refractory or resistant CMV infection.

2 | Methods

2.1 | Study Cohort and Design

This retrospective single-center cohort study included all lung transplant recipients who received letermovir for the treatment of refractory or resistant (R/R) CMV infection and/or CMV disease at the University of Munich, Germany, between March 2018 and August 2024. The study protocol was approved by the local ethics committee (UE No. 19–060), which waived the requirement for written informed consent because of the retrospective study design.

Eligible patients had undergone lung transplantation between February 2012 and October 2022. During this period, 749 lung transplant procedures were performed at our institution, representing the source population from which the study cohort was identified. Clinical records, virological findings, and treatment courses before and during letermovir therapy were reviewed retrospectively.

Initiation of rescue therapy with letermovir was determined by the treating transplant physician in patients with ganciclovir-resistant or refractory CMV infection. Refractory CMV infection was defined as failure to achieve a decline of at least 1 $\log_{10}$ in CMV DNA after ≥ 14 days of appropriately dosed antiviral therapy [16]. Resistant CMV infection at study entry was defined by the detection of resistance-associated mutations conferring reduced susceptibility to ganciclovir. Because letermovir was administered off-label for treatment of active CMV infection, reimbursement was requested from and approved by the respective health insurance provider before each treatment course.

Patients were subsequently stratified according to the occurrence of genotypically confirmed letermovir resistance and assigned to either the LET-resistance or absence of LET-resistance group. Letermovir resistance was defined by the detection of estab-

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lished CMV mutations associated with reduced susceptibility to letermovir. Among patients with available genotypic resistance testing prior to letermovir initiation, no pre-existing letermovir resistance was detected.

Virological response was defined as a reduction in CMV DNA of at least 1 $\log_{10}$, followed by complete viral clearance within eight weeks after initiation of letermovir. Patients were classified as treatment failures ("non-responders") if one or more of the following criteria were met: (i) failure to achieve a $\geq$1 $\log_{10}$ reduction in CMV DNA after four weeks of therapy, (ii) persistence of CMV DNAemia beyond week 8, or (iii) an increase in CMV DNA of at least 1 $\log_{10}$ during treatment. Virological clearance was defined as a plasma CMV DNA concentration below the lower limit of quantification of the assay (< 32 IU/mL). Both undetectable CMV DNA and detectable but non-quantifiable CMV DNA (< 32 IU/mL) were considered evidence of viral clearance. Sustained clearance required confirmation by two consecutive plasma samples obtained at least three days apart.

2.2 | Antiviral Prophylaxis and Therapy

Following lung transplantation, maintenance immunosuppression routinely consisted of a calcineurin inhibitor (tacrolimus or ciclosporin A), mycophenolate mofetil (MMF), and prednisone. Induction therapy was not part of the standard immunosuppressive protocol but was reserved for selected patients, including retransplant recipients and individuals with preformed donor-specific HLA antibodies.

CMV prophylaxis was administered according to donor and recipient CMV serostatus. Recipients at high risk for CMV infection (D+/R−) received valganciclovir prophylaxis for 9–12 months, whereas intermediate-risk patients (D+/R+ or D−/R+) were treated for 6 months and low-risk recipients (D−/R−) for 3 months. There was no predefined risk-stratification protocol to determine prophylaxis duration within the D+/R− group; rather, duration was individualized according to clinical factors, including renal function, hematologic toxicity/bone marrow suppression, and the preceding CMV course. Valganciclovir was prescribed at 900 mg once daily, with dose adjustments based on renal function. In patients who developed clinically relevant neutropenia during valganciclovir prophylaxis, valganciclovir was discontinued and a pre-emptive CMV monitoring strategy was applied. None of the patients received letermovir as primary CMV prophylaxis.

Patients who developed clinically relevant CMV DNAemia while receiving prophylaxis were switched to therapeutic-dose valganciclovir (900 mg twice daily, adjusted for renal function). Escalation of antiviral therapy was not based on a predefined CMV DNA threshold but on confirmation of clinically significant viremia in at least two consecutive plasma CMV-PCR measurements together with the overall clinical assessment by the treating transplant physician.

Patients with an insufficient virological response generally received intravenous ganciclovir (5 mg/kg every 12 h, adjusted for renal function) combined with CMV hyperimmune globulin (Cytotect; 100 IU/kg/day [1 mL/kg/day; 50 mg/kg/day]) for 5–7 days. Although foscarnet was used in selected patients, it was not routinely administered before initiation of letermovir because of concerns regarding its toxicity, particularly nephrotoxicity, in this vulnerable lung transplant population. In patients with persistent refractory or resistant CMV infection, rescue treatment with letermovir was considered as previously reported [11]. Cidofovir was not used for the treatment of R/R CMV at our center during the study period.

Following regulatory approval, maribavir became available for the treatment of refractory or resistant CMV infection and was administered orally at the approved dosage of 400 mg twice daily [6]. Nevertheless, its clinical use during 2023 and the first half of 2024 remained restricted because access depended on procurement through an international pharmacy and was further limited by temporary supply shortages and institutional resource constraints.

In selected patients, immunosuppressive therapy was modified to facilitate CMV control by conversion to an mTOR inhibitor-based quadruple regimen combined with low-dose tacrolimus, targeting trough concentrations of 3–5 ng/mL for both agents within one week after treatment modification. Depending on the clinical situation, the MMF dose was reduced or temporarily withheld, whereas the maintenance dose of prednisone was not routinely adjusted because of CMV infection.

Letermovir was administered orally at the approved prophylactic dose of 480 mg once daily or 240 mg once daily in patients receiving concomitant ciclosporin, in accordance with the approved prescribing information for CMV prophylaxis after allogeneic HSCT [6].

2.3 | CMV Monitoring

Recipient and donor CMV serostatus was determined before transplantation by measurement of anti-CMV IgG using automated serological assays. After lung transplantation, CMV surveillance was performed using quantitative CMV PCR in EDTA plasma twice weekly throughout the initial hospital stay. Following discharge, patients underwent weekly CMV DNA monitoring together with tacrolimus trough level measurements during the first two years after transplantation. Thereafter, CMV surveillance was continued lifelong, with testing intervals gradually extended to every two, three, and subsequently four weeks according to the patient's clinical course and individual risk of CMV reactivation. Accordingly, the minimum three-day interval used to define sustained clearance primarily reflected the more frequent CMV-PCR monitoring during hospitalization, whereas intervals between consecutive measurements were generally longer in the outpatient setting. All virological results were reviewed by transplant physicians at our center. Routine follow-up visits were scheduled at least every three months during the first two years after transplantation, with additional outpatient assessments or hospital admissions arranged whenever clinically indicated.

CMV DNA was quantified in EDTA plasma using the cobas CMV Test (Roche Diagnostics), a quantitative real-time polymerase chain reaction assay performed on the COBAS

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AmpliPrep/COBAS TaqMan platform. The assay has a lower limit of detection of approximately 32 IU/mL (35 copies/mL) [17].

Assessment of CMV-specific cell-mediated immunity (CMI) was performed using the T-Track CMV assay (Lophius Biosciences GmbH, Regensburg, Germany) according to the manufacturer's recommendations whenever requested by the treating physician. The results were used to evaluate recovery of CMV-specific immune function and to support individualized decisions regarding antiviral treatment duration and the likelihood of sustained virological control. As CMI testing is not part of routine post-transplant care at our institution and requires dedicated laboratory processing, analyses were not scheduled at predefined intervals. Whenever possible, samples were obtained before initiation and/or before discontinuation of antiviral therapy. A positive CMI response was defined as an interferon-γ response of $>$ 10 spots following stimulation with either IE-1 or pp65 CMV peptides [18].

CMV end-organ disease was diagnosed on the basis of histopathological examination of tissue biopsies or analysis of body fluid specimens obtained according to the clinical presentation. Proven CMV disease required demonstration of characteristic CMV-induced cytopathic changes together with positive immunohistochemical staining [16]. CMV central nervous system disease was diagnosed when CMV DNA was detected in cerebrospinal fluid in patients with compatible neurological symptoms and no evidence of clinically relevant blood contamination. Assessment of blood contamination was based on routine cerebrospinal fluid analysis in conjunction with the overall clinical context rather than on a predefined erythrocyte threshold [2].

2.4 | Genotype Testing

Genotypic antiviral resistance testing was performed whenever antiviral resistance was clinically suspected, as determined by the treating transplant physician.

Resistance analysis for ganciclovir, foscarnet, cidofovir, letermovir, and maribavir was carried out by PCR amplification followed by Sanger sequencing of the relevant regions of the UL97 (amino acids 312–666), UL54 (amino acids 265–1053), UL56 (amino acids 12–393), UL51, and UL89 genes. Sequencing was performed by Eurofins Genomics (Ebersberg, Germany), whereas sequence interpretation and identification of resistance-associated variants were undertaken at the Max von Pettenkofer Institute and the Gene Center, LMU Munich. Detected sequence variants were interpreted using published CMV resistance mutation databases and current international consensus recommendations [9, 12, 13, 19]. Phenotypic susceptibility testing, including determination of the 50% effective concentration (EC_{50}) required to inhibit viral replication and adjustment of antiviral therapy according to the degree of phenotypic resistance, was not performed.

2.5 | Statistical Analysis

Continuous variables are reported as mean ± standard deviation or median with interquartile range (Q1–Q3), whereas categorical variables are presented as absolute numbers and percentages. Comparisons of continuous variables between groups were performed using the Mann–Whitney U test. Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test, as appropriate. Variables associated with the emergence of letermovir resistance were evaluated by multivariable linear regression analysis. Statistical analyses were performed using IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA).

3 | Results

3.1 | Study Population

A total of 61 consecutive lung transplant recipients who were treated with letermovir for R/R CMV infection from March 2018 to August 2024 were assessed for their treatment response. CMV disease was present in 25 patients (41.0%) at the time of switch to letermovir as summarized in Table 1. 4 LTx recipients (6.6%) had multiple CMV end-organ manifestations involving more than one organ system. All patients were at least once admitted to our hospital for i.v. ganciclovir ± CMVIgG before rescue therapy with letermovir was initiated. Following initial treatment with i.v. ganciclovir with or without CMVIgG, 12 patients (19.7%) received additional sequential treatment for R/R CMV infection before initiation of letermovir, including foscarnet in 10 patients (16.4%), maribavir in one patient (1.6%), and leflunomide in one patient (1.6%) (Table S1). During the study period, 22 additional patients with R/R CMV who did not receive letermovir achieved CMV DNAemia clearance with alternative antiviral treatment, including maribavir ($n = 16$) and foscarnet ($n = 6$). Prior to letermovir initiation, ganciclovir resistance was detected in 28 patients (45.9%), cidofovir resistance in 9 patients (14.8%), foscarnet resistance in 4 patients (6.6%), and maribavir resistance in 1 patient (1.6%) (Table 2). None of the patients in the study cohort received induction therapy. Demographics and characteristics of the study population are shown in Table 1.

3.2 | Letermovir, Concomitant Therapy, and CMV-Specific Cell-Mediated Immunity

In most of the patients (60.7%) monotherapy with letermovir was initiated and no further action was taken. In 24 LTx recipients (39.3%) one or more additional antiviral interventions were performed simultaneously (± 4 weeks) to initiation of letermovir. Simultaneous interventions included (val)ganciclovir administration ($n = 11$), foscarnet administration ($n = 3$), combined (val)ganciclovir and CMVIgG administration ($n = 8$), combined (val)ganciclovir, foscarnet and CMVIgG administration ($n = 2$). In addition, in two patients, a switch to an mTOR-inhibitor-based quadruple low tacrolimus immunosuppression regimen was performed after the initiation of letermovir.

CMV-specific T-cells directed to CMV-IE1 and CMV-pp65 were measured by interferon-γ Elispot assay at a median of 73.5 days [range: 1–285] after initiation of rescue therapy with letermovir. Before initiation of letermovir, CMV-specific immunity testing was performed in 16 of 61 patients (26.2%), with five patients demonstrating a positive response despite uncontrolled CMV infection. During letermovir treatment, 45 CMV-specific immunity tests were performed in 42 of 61 patients (68.9%),

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TABLE 1 | Demographics and characteristics of the study cohort.

	All ($n = 61$)
Age (years)	57.3 ± 8.8
Sex (male), *n* (%)	34 (55.7)
BMI (kg/m^2)	22.6 ± 3.9
Type of transplant, *n* (%)	
Bilateral lung transplantation (BLTx)	56 (91.8)
Underlying diseases	
COPD, *n* (%)	21 (34.4)
ILD, *n* (%)	31 (50.8)
CF, *n* (%)	6 (9.8)
Others, *n* (%)	3 (4.9)
CMV-serostatus	
D+/R−	51 (83.6)
D+/R+	7 (11.5)
D−/R+	2 (3.3)
D−/ R−	1 (1.6)
Maintenance immunosuppression[a], *n* (%)	
FK-506 + MMF + prednisone	44 (72.1)
CsA + MMF + prednisone	6 (9.8)
FK-506 +MMF + prednisone + mTOR[b]	11 (18.1)
CMV disease: organs, *n* (%)/patients, *n* (%)	30 (49.2)/25 (41.0)
CMV pneumonitis	8 (13.1)
CMV gastritis/esophagitis	10 (16.4)
CMV colitis	10 (16.4)
CMV retinitis	1 (1.6)
CMV encephalitis	1 (1.6)

Note: Data are presented as mean ± standard deviation and number and percentage, respectively. FK-506 = tacrolimus.

Abbreviations: BLTx, bilateral lung transplantation; BMI, body mass index; CF, cystic fibrosis; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CsA, ciclosporin A; ILD, interstitial lung diseases; LTx, lung transplantation; MMF, mycophenolate mofetil.

[a]Immunosuppression before initiation of rescue therapy.

[b]Everolimus or Sirolimus.

including 14 patients who developed letermovir resistance and 28 patients who did not. Overall, 13 of the 45 tests performed during letermovir treatment were positive (Table 2). Time from initiation of letermovir and assessment of CMV-specific T-cell-immunity was 66 [1–284] and 96 [26–179] days in patients with negative and positive results, respectively. Time from initiation of letermovir and assessment of CMV-specific T-cell-immunity was 61.5 [1–284] and 79 [25–171] days in patients who did not develop letermovir resistance and those who did, respectively.

3.3 | Efficacy of Letermovir and Resistance

The course of CMV viral load before and after initiation of letermovir is depicted in Figure 1. Rescue therapy with letermovir was initiated at a median of 10.3 [1.2–87.6] months after LTx and applied for a median of 127 [48–603] days. In case of suspicion of treatment failure resistance testing was performed in 21 patients (34.4%) on request of the treating physician. In 19 patients (31.1%) a mutation conferring letermovir resistance was detected 96 [51–395] days after initiation. Most resistance mutations involved codon 325 of the UL56 gene (15/19, 78.9%), with C325Y and C325F being the predominant variants. All 19 patients harbored established UL56 mutations known to confer letermovir resistance. Two patients additionally carried the UL56 substitutions Y321C and T244K, which have been associated with only low-level reductions in letermovir susceptibility (< 5-fold increase in EC50) and are generally not considered clinically relevant at therapeutic drug concentrations. One patient additionally harbored the UL56 T189M substitution, a sequence variant that has been reported as a genetic polymorphism and has not been associated with phenotypically confirmed letermovir resistance. Letermovir resistance emerged in 4 out of 19 patients during the second round of letermovir treatment. Detailed information about the mutations is provided in the supplementary material (Table S2). Among the 19 patients who developed letermovir resistance, 11 patients (57.9%) ultimately achieved complete CMV DNA clearance following modification or intensification of antiviral therapy (switch to or add-on of [val]ganciclovir, $n = 6$; switch to maribavir, $n = 4$; and continued letermovir treatment, $n = 1$). Five patients (26.3%) never achieved complete clearance but subsequently maintained persistent low-level CMV DNAemia (100–5.000 IU/mL), including four patients receiving maribavir-based salvage therapy and one patient managed with repeated intravenous immunoglobulin. The remaining three patients experienced progressive CMV DNAemia despite multiple salvage treatment strategies. Salvage therapies included foscarnet plus CMV hyperimmune globulin (Cytotect) ($n = 1$), a sequential regimen of foscarnet followed by maribavir and CMV-specific T-cell therapy ($n = 1$), and a sequential regimen of maribavir followed by foscarnet ($n = 1$).

Of the remaining 40 patients (65.6%), CMV viral load decreased significantly (> 1 log) after a median of 14.5 [5–31] days and was subsequently cleared after a median of 34 [6–126] days. Following documented virologic clearance, letermovir was continued as secondary prophylaxis in all 40 patients for a median of 67.5 [47–113] days. During ongoing letermovir secondary prophylaxis, breakthrough CMV DNAemia occurred in one patient (2.5%) after 120 days. After discontinuation of letermovir, 13 additional patients developed recurrent CMV infection after a median of 73 [43–210] days from the last negative CMV PCR. In these patients, letermovir had been continued for a median of 51 [40–83.5] days after virologic clearance. At recurrence, the median CMV viral load was 2.040 IU/mL [range 561–8.836]. Of these 13 patients, 10 were retreated with letermovir, resulting in successful viral clearance in all 10 cases.

LTx recipients who developed letermovir resistance had a significantly higher median viral load at initiation of rescue therapy (35.100 [13.900–228.000] vs. 7.805 [1.173–30.350] IU/mL, $p = 0.018$) and a lower rate of positive CMV-specific cell-mediated immunity responses (7.1% vs. 38.7%; $p = 0.038$), as shown in Table 2. In addition, time to a significant (≥ 1 log 10) decrease of viral load was shorter in patients who did not develop letermovir resistance (16 [5–31] vs. 34 [17–58] days, $p < 0.001$).

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TABLE 2 | Baseline antiviral resistance and clinical characteristics according to subsequent development of letermovir resistance.

	All ($n = 61$)	LET-Resistance ($n = 19$)	Absence of LET-resistance ($n = 42$)	p-value
Age (years)	57.3 ± 8.8	61.2 ± 8.3	60.9 ± 5.5	0.051
Sex (male), *n* (%)	34 (55.7)	11 (57.9)	23 (54.8)	1.000
BMI (kg/m^2)	22.6 ± 3.9	23.2 ± 4.0	22.3 ± 3.9	0.435
Ganciclovir-resistance, *n* (%)[a]	28 (45.9)	12 (63.2)	16 (38.1)	0.737
UL 97 gene mutation, *n* (%)	18 (64.3)	4 (22.2)	14 (63.6)	**0.025**
UL 54 gene mutation, *n* (%)	2 (7.1)	2 (11.1)	0 (0.0)	0.487
UL 97 + UL 54 gene mutation, *n* (%)	8 (28.6)	6 (33.3)	2 (9.1)	0.235
Cidofovir-resistance, *n* (%)[a]	9 (14.8)	7 (38.9)	2 (9.1)	0.057
Foscarnet-resistance, *n* (%)[a]	4 (6.6)	4 (22.2)	0 (0.0)	**0.038**
Maribavir-resistance, *n* (%)[a]	1 (1.6)	0 (0.0)	1 (2.4)	1.000
Number of CMV-reactivations before rescue therapy	2.3 ± 1.1	2.1 ± 1.0	2.4 ± 1.3	0.288
Time until letermovir treatment after LTx (months)	18.5 ± 22.1	9.7 ± 4.9	22.5 ± 25.6	**0.035**
CMV viral load (IU/mL) at initiation of letermovir, median [IQR]	13.400 [2.535–64.450]	35.100 [13.900–228.000]	7.805 [1.173–30.350]	**0.018**
CMV viral load > 10.000 IU/mL at initiation of letermovir, *n* (%)	32 (52.5)	18 (94.7)	14 (33.3)	**< 0.001**
Duration of therapy with letermovir (days)[b]	182.6 ± 139.4	224.0 ± 147.4	163.5 ± 133.0	0.137
CMV-specific cell-mediated immunity tests during/after LET, *n* positive /performed tests (%)[c]	13/45 (28.9)	1/14 (7.1)	12/31 (38.7)	**0.038**
Time to CMV-CMI test after initiation of LET (weeks)	13.5 ± 10.2	12.5 ± 5.9	13.9 ±11.8	0.661
Presence of CMV organ disease, *n* (%)	25 (41.0)	10 (52.6)	15 (35.7)	0.266
Antiviral monotherapy with letermovir, *n* (%)	37 (60.7)	9 (47.4)	28 (66.7)	0.154
Time to decrease of 1 $\log_{10}$ after initiation of letermovir (days)	21.5 ± 11.5	33.9 ± 10.7	15.8 ± 6.2	**< 0.001**

Note: Continuous data are presented as mean ± standard deviation or median [interquartile range], as appropriate; categorical data are presented as number and percentage.

Abbreviations: BMI, body mass index; CMV, cytomegalovirus; CMV-CMI, CMV-specific cell-mediated immunity; LET, letermovir.

[a]Tested in 40/61 patients.

[b]Cumulative time; one patient is still on therapy with letermovir.

[c]45 CMV-specific immunity tests were available during or after letermovir treatment, performed in 42 patients (14/28). CMV-specific T cells directed to CMV-IE1 and CMV-pp65 were measured by interferon-γ Elispot assay; cut-off values were >10 spots and > 10 spots, for the pp65 and IE1 ELISPOT assays, respectively.

In multivariable regression analysis time to decrease of 1 $\log_{10}$ (OR 0.721, CI 0.368–0.857; $p < 0.001$) after initiation of letermovir and CMV viral load before rescue therapy (OR 1.110, CI 0.880–1.250; $p = 0.044$) were independently associated with a higher risk of development resistance while controlling for age, sex, BMI, number of CMV-reactivations before rescue therapy, presence of CMV disease, antiviral monotherapy with letermovir, duration of rescue therapy (Table 3).

4 | Discussion

Resistant or refractory CMV infection remains a major challenge in post-transplant CMV management [2]. Hence, knowledge of drug efficacy is crucial for an adequate choice of antiviral medication, carefully balancing antiviral potency versus the risk of harmful side effects and antiviral resistance. Recently, two new antiviral drugs, letermovir and maribavir, became available. The side effect profile has been superior compared to conventional drugs, however, both letermovir and maribavir indicate a low viral genetic barrier to resistance [9, 10, 20, 21]. Data on the rates and risk factors associated with letermovir resistance in lung transplant recipients are scarce [11, 20, 22].

In our study, letermovir was applied as described for the prophylaxis in HSCT recipients but showed therapeutic potential in R/R CMV infection in the majority of the patients [6, 10]. Letermovir was used alone or alongside CMV IgG and immunosuppressive adjustments. Therefore, the effect on CMV viral load cannot be solely attributed to letermovir and it must be viewed in the context of several previous and simultaneous antiviral interventions. In patients responding to letermovir, CMV viral load decreased significantly (≥ 1 $\log_{10}$) within approximately 2–3 weeks and was subsequently cleared. Therefore, a four-

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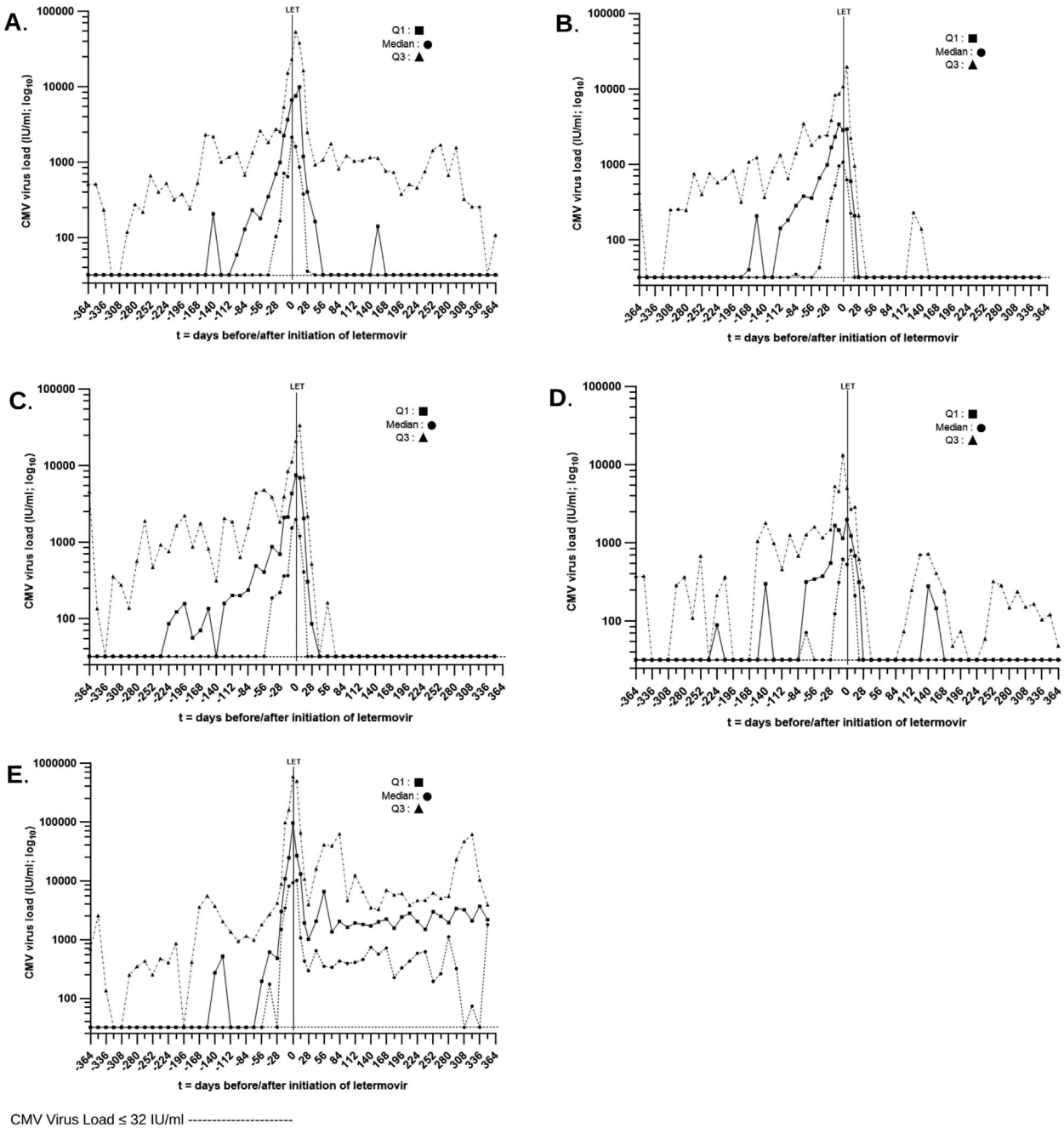


FIGURE 1 | Course of CMV viral load before and after initiation of letemovir. (A) all patients ($n = 61$); (B) all responders ($n = 40$); (C) responder without relapse ($n = 27$); (D) responder who developed letermovir resistance ($n = 13$); (E) patients with resistance ($n = 19$). Data are provided as mean and Quartile 1 and 3. The horizontal dashed line indicates the threshold for quantitative detection of CMV plasma DNA by the assays used in this series (32 IU/mL).

week interval may be sufficient to evaluate antiviral activity in most patients. While contemporary CMV guidelines emphasize virologic response rather than time-based response criteria, this observation reflects the viral kinetic patterns observed in our cohort rather than a proposed treatment endpoint [2]. It remains unclear whether this observation can be attributed to the novel mode of action of letermovir. Markers for active viral replication, such as CMV pp65, might be a more suitable tool to assess early treatment response. In addition, CMV relapse after letermovir discontinuation could be successfully retreated with letermovir in 71.4% of the cases.

In a multicenter observational study by Linder et al., patients who initiated letermovir at CMV viral loads < 1000 IU/mL ($n = 37$) achieved favorable virologic outcomes, whereas outcomes were less favorable when treatment was started at higher viral loads [22]. This observation is also confirmed in our study cohort, where patients with a viral load > 10.000 IU/mL experienced

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TABLE 3 | Multivariate linear regression analysis (MLR, OR) to identify risk factors associated with evolution of letermovir resistance.

	Letermovir resistance		
All patients ($n = 58$)	**MLR *p*-value**	**OR**	**95% CI**
Age (years)	0.551	−0.082	−0.020–0.011
Sex (male), *n* (%)	0.563	0.067	−0.153–0.277
BMI (kg/m^2)	0.983	−0.002	−0.031–0.030
Number of CMV-reactivations before rescue therapy	0.140	0.208	−0.029–0.297
CMV viral load (IU/mL) at initiation of letermovir	**0.044**	**1.110**	0.880–1.250
Duration of therapy with letermovir (days)[a]	0.550	0.074	−0.001–0.001
Positive CMV-specific cell-mediated immunity during LET	0.116	−0.223	−0.516–0.059
CMV disease, *n* (%)	0.829	0.025	−0.192–0.238
Antiviral monotherapy with letermovir	0.726	−0.043	−0.270–0.190
Time to decrease of 1 $\log_{10}$ after initiation of letermovir (days)	**0.000**	**0.721**	0.368–0.857

Abbreviations: BMI, body mass index; CMV, cytomegalovirus; CMV-CMI, CMV-specific cell-mediated immunity; LET, letermovir.
[a]Cumulative time; one patient is still on therapy with letermovir.

significantly higher failure rates to antiviral therapy. Whether combination therapy in patients with risk for failure of second line therapy is useful in a selected cohort of patients remains to be established.

Treatment failure to salvage therapy with letermovir was observed in approximately one-third of the patients. In 31.1% of the patients, a mutation conferring letermovir resistance was detected. Robust data on rates of letermovir resistance are not available, as the drug has not been used for treatment. When used for treatment, letermovir seems to have the lowest barrier, followed by maribavir and GCV/VGCV [3, 19, 20]. In a Phase 3 study comparing letermovir with valganciclovir for CMV prophylaxis in kidney transplant recipients, no specific data on the frequency of letermovir resistance in this patient group were reported [23]. Another study observed that CMV-seropositive patients receiving letermovir prophylaxis may still experience breakthrough viremia and develop reduced susceptibility to the drug. Notably, a limited resistance analysis from the trial identified a resistance-associated pUL56 V236M variant in one patient, raising concerns given the previously suggested low resistance threshold for letermovir in preclinical studies [6]. In a Phase 3 study comparing maribavir versus investigator assigned therapy for R/R CMV, the barrier for maribavir resistance seems similarly low (~ 25%), posing the risk for treatment failure [6].

Risk factors are not well defined. However, the most significant risk factor for resistant CMV across multiple trials is the lack of prior CMV immunity, seen in CMV mismatched D+/R− recipients. Other risk factors for development of resistance include inadequate antiviral drug dose or delivery, prolonged antiviral drug exposure (usually > 3 months), persistent active viral replication while on antiviral therapy, intense immunosuppressive therapy, and exposure to therapeutic antiviral drugs with a lower barrier to resistance [3, 10, 19, 20]. In our study, high viral load and slow treatment response were independently associated with the development of mutations conferring letermovir resistance. Antiviral drug resistance should be suspected when there is recurrent CMV DNAemia or disease during prolonged antiviral therapy [2]. Our data suggest that clinicians should be aware that the kinetics of CMV DNAemia response and the risk for early emergence of resistance may be different with newer antiviral drugs, especially those that have a lower barrier to resistance. Genetic analysis is more feasible and reliable if the CMV DNAemia is at least 1000 IU/mL [24]. Therefore, we recommend to perform sequencing, when time to a significant (≥ 1 $\log_{10}$) decrease of viral load is longer than 14 days and viral load is ≥ 1000 IU/mL.

Optimal duration of letermovir use upon viral clearance has to be assessed. Treatment duration varied in our study, which was based on insurance coverage and clinical grounds. In all patients responding to letermovir, treatment was continued until clearance of CMV viral load and was continued for a median of approximately 2 months after the first negative CMV- PCR. When ultrasensitive CMV DNAemia assays are used, current guidelines recommend continuing antiviral treatment until one or two negative or very low CMV DNAemia results are obtained one week apart [2]. In patients receiving letermovir as rescue therapy, prolonged exposure in the setting of ongoing viral replication should be avoided given the risk of treatment-emergent resistance. However, resistance rarely occurs after brief exposure to antiviral therapy [3, 4]. Patients responding to letermovir exhibited higher CMV-specific T-cell immunity responses. Immune monitoring of CMV-specific T-cell responses can predict individuals at increased risk for CMV infection and relapse and might be useful in guiding duration of rescue therapy with letermovir [2, 18].

Several limitations should be considered when interpreting our results, including the retrospective, single-center design and the absence of a control group. Furthermore, as letermovir treatment required prior approval by the respective health insurance provider, treatment allocation may have been subject to selection bias, and the study cohort may therefore not fully represent all lung transplant recipients with R/R CMV infection treated at our center. Moreover, definitions of virological response to CMV treatment are not fully standardized across the literature,

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and the predefined response criteria used in our study may therefore limit comparability with other studies. In addition, the cohort was characterized by considerable heterogeneity in CMV management strategies, reflecting changes in clinical practice and evolving treatment approaches over the study period. Although maribavir became available during the study period, its use was limited by restricted availability and supply shortages. Consequently, only one patient received maribavir, precluding a meaningful comparison with letermovir. Nevertheless, because prospective controlled data on therapeutic letermovir remain scarce and its use for active CMV infection is still largely off-label, real-world observational studies are essential to inform clinical decision-making and optimize patient selection for this therapeutic strategy.

In conclusion, our findings highlight the substantial risk of treatment-emergent resistance when letermovir is used for active R/R CMV infection, particularly in patients with high baseline viral loads and a slow virological response. These findings support the established role of letermovir primarily for CMV prophylaxis and suggest that its use as monotherapy for active R/R CMV infection should be approached cautiously, particularly in patients with high-level DNAemia. In patients with low viral loads or when other established treatment options are unavailable or limited by toxicity or resistance, letermovir may remain a therapeutic option with close virological monitoring. For patients with high viral loads, alternative strategies, including initial viral load reduction with other active antiviral agents before switching to letermovir or combination approaches, may be considered, although these strategies require further prospective evaluation.

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Data Availability Statement

Data are available on request from the authors.

References

1. M. Kawashima, J. Ma, E. Huszti, et al., "Association Between Cytomegalovirus Viremia and Long-Term Outcomes in Lung Transplant Recipients," *American Journal of Transplantation* 24, no. 6 (2024): 1057–1069, https://doi.org/10.1016/j.ajt.2024.01.027.

2. C. N. Kotton, D. Kumar, O. Manuel, et al., "The Fourth International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation," *Transplantation* 109, no. 7 (2025): 1066–1110, https://doi.org/10.1097/TP.0000000000005374.

3. C. E. Fisher, J. L. Knudsen, E. D. Lease, et al., "Risk Factors and Outcomes of Ganciclovir-Resistant Cytomegalovirus Infection in Solid Organ Transplant Recipients," *Clinical Infectious Diseases* 65, no. 1 (2017): 57–63, https://doi.org/10.1093/cid/cix259.

4. S. Hantz, F. Garnier-Geoffroy, M.-C. Mazeron, et al., "Drug-Resistant Cytomegalovirus in Transplant Recipients: A French Cohort Study," *Journal of Antimicrobial Chemotherapy* 65, no. 12 (2010): 2628–2640, https://doi.org/10.1093/jac/dkq368.

5. A. P. Limaye, K. Budde, A. Humar, et al., "Letermovir vs Valganciclovir for Prophylaxis of Cytomegalovirus in High-Risk Kidney Transplant Recipients," *Jama* 330, no. 1 (2023): 33, https://doi.org/10.1001/jama.2023.9106.

6. F. M. Marty, P. Ljungman, R. F. Chemaly, et al., "Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation," *New England Journal of Medicine* 377, no. 25 (2017): 2433–2444, https://doi.org/10.1056/NEJMoa1706640.

7. J. Piret and G. Boivin, "Clinical Development of Letermovir and Maribavir: Overview of Human Cytomegalovirus Drug Resistance," *Antiviral Research* 163 (2019): 91–105, https://doi.org/10.1016/j.antiviral.2019.01.011.

8. R. F. Chemaly, S. Chou, H. Einsele, et al., "Definitions of Resistant and Refractory Cytomegalovirus Infection and Disease in Transplant Recipients for Use in Clinical Trials," *Clinical Infectious Diseases* 68, no. 8 (2019): 1420–1426, https://doi.org/10.1093/cid/ciy696.

9. S. Chou, "Rapid in Vitro Evolution of Human Cytomegalovirus UL56 Mutations That Confer Letermovir Resistance," *Antimicrobial Agents and Chemotherapy* 59, no. 10 (2015): 6588–6593, https://doi.org/10.1128/AAC.01623-15.

10. R. R. Razonable, "Current Perspectives on Letermovir and Maribavir for the Management of Cytomegalovirus Infection in Solid Organ Transplant Recipients," *Drug Design, Development and Therapy* 18 (2024): 3987–4001, https://doi.org/10.2147/DDDT.S265644.

11. T. Veit, D. Munker, J. Barton, et al., "Letermovir in Lung Transplant Recipients With Cytomegalovirus Infection: A Retrospective Observational Study," *American Journal of Transplantation* 21, no. 10 (2021): 3449–3455, https://doi.org/10.1111/ajt.16718.

12. S. Chou, "Approach to Drug-Resistant Cytomegalovirus in Transplant Recipients," *Current Opinion in Infectious Diseases* 28, no. 4 (2015): 293–299, https://doi.org/10.1097/QCO.0000000000000170.

13. F. Horsten, S. Gillemot, Calò, et al., "Cytomegalovirus Infection and Drug Resistance Emergence During Letermovir Salvage Therapy in a Pediatric SCID Patient," *npj Antimicrobials and Resistance* 3, no. 1 (2025): 43, https://doi.org/10.1038/s44259-025-00118-y.

14. E. von Hoerschelmann, J. Münch, L. Gao, et al., "Letermovir Rescue Therapy in Kidney Transplant Recipients With Refractory/Resistant CMV Disease," *Journal of Clinical Medicine* 13, no. 1 (2023): 100.

15. H. N. Imlay and D. R. Kaul, "Letermovir and Maribavir for the Treatment and Prevention of Cytomegalovirus Infection in Solid Organ and Stem Cell Transplant Recipients," *Clinical Infectious Diseases* 73, no. 1 (2021): 156–160, https://doi.org/10.1093/cid/ciaa1713.

16. P. Ljungman, R. F. Chemaly, F. Khawaya, et al., "Consensus Definitions of Cytomegalovirus (CMV) Infection and Disease in Transplant Patients Including Resistant and Refractory CMV for Use in Clinical Trials: 2024 Update from the Transplant Associated Virus Infections Forum," *Clinical Infectious Diseases* 79, no. 3 (2024): 787–794, https://doi.org/10.1093/cid/ciae321.

17. H. H. Hirsch, I. Lautenschlager, B. A. Pinsky, et al., "An International Multicenter Performance Analysis of Cytomegalovirus Load Tests," *Clinical Infectious Diseases* 56, no. 3 (2013): 367–373, https://doi.org/10.1093/cid/cis900.

18. T. Veit, M. Pan, D. Munker, et al., "Association of CMV-Specific T-Cell Immunity and Risk of CMV Infection in Lung Transplant Recipients," *Clinical Transplantation* 35, no. 6 (2021): e14294, https://doi.org/10.1111/ctr.14294.

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19. N. S. Lurain and S. Chou, "Antiviral Drug Resistance of human cytomegalovirus," *Clinical Microbiology Reviews* 23, no. 4 (2010): 689–712, https://doi.org/10.1128/CMR.00009-10.

20. L. Cherrier, A. Nasar, K. J. Goodlet, M. D. Nailor, S. Tokman, and S. Chou, "Emergence of Letermovir Resistance in a Lung Transplant Recipient With Ganciclovir-Resistant Cytomegalovirus Infection," *American Journal of Transplantation* 18, no. 12 (2018): 3060–3064, https://doi.org/10.1111/ajt.15135.

21. S. Chou, D. J. Winston, R. K. Avery, et al., "Comparative Emergence of Maribavir and Ganciclovir Resistance in a Randomized Phase 3 Clinical Trial for Treatment of Cytomegalovirus Infection," *Journal of Infectious Diseases* 231, no. 3 (2025): e470–e477, https://doi.org/10.1093/infdis/jiae469.

22. K. A. Linder, C. Kovacs, K. M. Mullane, et al., "Letermovir Treatment of Cytomegalovirus Infection or Disease in Solid Organ and Hematopoietic Cell Transplant Recipients," *Transplant Infectious Disease* 23, no. 4 (2021): e13687, https://doi.org/10.1111/tid.13687.

23. J. M. Strizki, T. L. Diamond, V. L. Teal, et al., "Cytomegalovirus Antiviral Resistance among Kidney Transplant Recipients in a Phase 3 Trial of Letermovir vs Valganciclovir Prophylaxis," *Journal of Infectious Diseases* 230, no. 6 (2024): e1287–e1298, https://doi.org/10.1093/infdis/jiae287.

24. M. K. Sahoo, M. I. Lefterova, F. Yamamoto, et al., "Detection of cytomegalovirus Drug Resistance Mutations by next-generation Sequencing," *Journal of Clinical Microbiology* 51, no. 11 (2013): 3700–3710, https://doi.org/10.1128/JCM.01605-13.

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