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Emergence of letermovir resistance in solid organ transplant recipients with ganciclovir resistant cytomegalovirus infection: A case series and review of the literature

Running title: Letermovir resistance in SOT

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ABSTRACT

Ganciclovir (GCV) resistant cytomegalovirus (CMV) infection is a common problem among solid organ transplant (SOT) recipients without prior CMV immunity (CMV D+/R-). GCV resistant CMV represents a particular challenge for CMV management. Letermovir is a recently licensed antiviral agent for primary CMV prophylaxis in allogeneic hematopoietic stem cell transplant (HSCT) recipients. Given the favorable safety profile and its oral bioavailability letermovir may be considered a valuable off-label option for secondary prophylaxis of GCV resistant CMV in SOT recipients. Here, we describe our experience with letermovir as secondary prophylaxis for GCV resistant CMV in two renal transplant recipients and review the literature in regard of previously published cases. Letermovir resistance emerged after a few months of secondary prophylaxis in the two renal transplant recipients. In both cases, the previously described *UL56 C325Y* letermovir resistance mutation was detected. *In vitro* studies of letermovir suggest a relatively low genetic barrier to resistance. Therefore, caution is warranted when using letermovir as secondary prophylaxis for GCV resistant CMV infection.

Key Words: Letermovir resistance, solid organ transplant, human cytomegalovirus infection (CMV), secondary CMV prophylaxis

INTRODUCTION

Human cytomegalovirus (CMV) infection remains an important contributor to morbidity after solid organ transplantation (SOT).¹ Given the high frequency of CMV primary infection and reactivation after SOT, adequate prevention strategies are important as they contribute to clinical transplant success, by decreasing the risk of CMV disease. Both, universal antiviral prophylaxis and preemptive therapy are recommended approaches to prevent CMV disease.² Nevertheless, secondary prophylaxis following preemptive therapy is used by many clinicians in the hope of reducing the risk of relapse.³ However, there are no randomized controlled trials of secondary prophylaxis for prevention of recurrent CMV infection in SOT recipients following resolution of CMV infection or disease, and most observational studies show no clear benefit of this strategy.^{4,5}

SOT recipients with (val)ganciclovir resistant CMV and without sufficient immunological CMV control following initial treatment and reduction in immunosuppression, represent a particular challenge for CMV management. Foscarnet salvage therapy is often successful in these situations but metabolic and renal toxicity and the need for intravenous administration complicate a prolonged therapy or secondary prophylaxis.^{6,7} Letermovir is a recently licensed antiviral agent, which was approved in 2017 for primary CMV prophylaxis in adult CMV-seropositive allogeneic hematopoietic stem cell transplant (HSCT) recipients.⁸ Due to the novel mechanism of action of letermovir (inhibition of the CMV terminase complex), cross-resistance with CMV polymerase inhibitors ((val)ganciclovir, cidofovir or foscarnet) does not occur.⁹ Given its favorable safety profile and oral bioavailability, letermovir may be considered a valuable option for off-label secondary prophylaxis or treatment of CMV that has become resistant to DNA polymerase inhibitors.¹⁰⁻¹²

Here, we describe our experience with letermovir secondary prophylaxis for (val)ganciclovir resistant CMV in two renal transplant recipients.

MATERIALS AND METHODS

CMV viral load (VL) was measured in EDTA plasma using quantitative CMV polymerase chain reaction (PCR) following the established laboratory developed test (LDT)-protocol targeting the *UL83* gene coding for the 65 kDa phosphoprotein of HCMV of the Institute for Infectious Diseases, University of Bern, Switzerland. The linear range of this assay is 500 to 10'000'000 cp/ml with an analytical sensitivity (limit of detection, LOD) of 250 cp/ml. Genotypic *UL97*, *UL54*, *UL51*, *UL56* and *UL89* CMV resistance testing (based on Sanger sequencing) was performed at the Institute for Infectious Diseases, University of Bern, Switzerland, using an in-house protocol (Supplemental material). The impact of specific genotypic resistance mutation on the susceptibility to antiviral drugs was reported as *resistance index* (RI). Resistance indices are defined as the mutant's drug specific EC50 (drug concentration needed to inhibit viral replication by 50%) divided by the wild-types EC50.¹³ For relevant CMV resistance mutations RIs have been published and made available for interpretation. We used published data for interpretation of CMV mutations and corresponding RIs.^{13,14}

CMV-specific cell mediated immunity (CMI) was measured using a commercially available IFN- γ ELISpot assay (T-Track[®] CMV, Lophius Biosciences, Regensburg, Germany) according to the manufacturer's instructions.¹⁵ A test was considered positive if the mean of four replicate square-root-transformed spot-forming cells (sqrt-SFC) for 200,000 cells (SRM) resulting from early-1 protein (IE-1) and/or pp65 stimulation was $\geq \text{sqrt}(10)$ and if the difference of the mean of sqrt-SFC of the stimulated condition to that of the unstimulated condition (SRM[stimulated] - SRM[unstimulated]) was ≥ 0.742 .¹⁶

CASE REPORTS

Case 1)

A 64-year old man underwent kidney transplant due to chronic urinary tract obstruction. Immunosuppression consisted of prednisone, mycophenolate mofetil, and tacrolimus. Basiliximab induction was used (Figure 1).

The donor was CMV seropositive and the recipient CMV seronegative (D+/R-). Valganciclovir was started with transplantation (dose adjusted for impaired renal function). Three month later, the patient developed primary breakthrough CMV infection. At that time, no CMV mutations associated with drug resistance in *UL97*, *UL54*, *UL56*, *UL89*, and *UL51* were detected and valganciclovir was continued in increased, therapeutic dosage. Despite an initial viral response and the presence of CMV-specific T-cells (30 SFC/2x10⁵ input cells, positive test result) the CMV VL increased (up to 16'700 cp/mL) during valganciclovir treatment and the patient developed persistent diarrhea. CMV resistance testing six months after transplantation revealed ganciclovir resistance (*UL97* mutations: *L595F*, *H520Q*, and *M460V* with ganciclovir resistance index (RI) =15.7, 10.0, and 9.8 respectively).^{13,17,18} Colonoscopy revealed histopathological changes compatible with mycophenolate mofetil (MMF) toxicity, CMV colitis could not be confirmed. In order to improve the immune function and due to MMF colitis the drug was discontinued. The patient refused intravenous foscarnet treatment and therefore valganciclovir was continued. Steadily increasing CMV VL (up to 91'000 copies/mL) triggered repeated CMV resistance testing nine months post-transplantation. Additional mutations associated with ganciclovir resistance (*UL54* mutation: *A987G*; ganciclovir RI=5.3, foscarnet RI=1.2) were detected.¹³ After developing a CMV viral syndrome (fatigue, progressive leukopenia) the patient agreed to intravenous foscarnet therapy (20mg per kg bid, estimated glomerular filtration rate [eGFR] 21 ml/min). Additionally, immunosuppression was changed; tacrolimus was replaced by cyclosporine due to increased tacrolimus trough levels in association with diarrhea.¹⁹ CMV viral load decreased to undetectable levels during therapy with foscarnet and the treatment was discontinued after eight weeks of treatment and one week of undetectable CMV viremia. In order to prevent CMV reactivation, letermovir (240 mg qd) was started as secondary prophylaxis. During follow up, low level viremia (up to 200 copies/mL) was intermittently detected. Progressive deterioration of the renal function, led to renal biopsy and revealed

cellular allograft rejection (Banff classification 1B). Rejection was treated with a short course of high-dose corticosteroids (methylprednisolone 500mg qd for three days, followed by tapered prednisone) and immunosuppression was intensified (mycophenolate mofetil resumed and dosage of cyclosporine A increased). Shortly after increasing the immunosuppression CMV viremia significantly increased and the patient developed severe fatigue without other signs of CMV viral syndrome. Repeated resistance testing revealed letermovir high-level resistance (*UL56* mutation *C325Y* RI >3000), a persistence of the *UL97* mutation *M460V*, and a new *UL54* mutation *P522S* (ganciclovir RI=3.1, foscarnet RI=1.1).^{13,14,20} Letermovir was discontinued, foscarnet (40 mg/kg bid, eGFR 20 ml/min) was initiated, and the immunosuppressive therapy was modified (low dose cyclosporine in combination with everolimus). In addition, CMV immune globulins were administered (despite missing clear evidence for benefit).¹ After the CMV VL dropped below the limit of detection for two weeks, foscarnet was discontinued. Subsequently, the CMV-specific CMI increased (as evidenced by a rise in pp65- and IE-1-specific T-cell response, Figure 1) and the patient was able to immunologically control CMV VL on a low level.

Case 2)

A 49 year old long term renal transplant recipient (transplantation 18 years earlier) with a follow up complicated by multiple rejection episodes was admitted to our outpatient service due to recurrent CMV infection. The donor was CMV seropositive and the recipient CMV seronegative (D+/R-). Maintenance immunosuppressive regimen consisted of cyclosporine, azathioprine and prednisone. Ganciclovir prophylaxis was given at renal transplantation, and followed by several courses of intravenous ganciclovir and long-term treatment with oral valganciclovir (Figure 2).

Asymptomatic CMV replication was detectable (10'400 copies/mL) despite ongoing valganciclovir treatment. Resistance testing detected a CMV *UL97* mutation (*C607F*, ganciclovir RI = 1.9).^{13,21} Since the mutation does not cause ganciclovir high-level resistance, intravenous high-dose ganciclovir (2.5 mg per kg bid, eGFR 25 ml/min) was administered and immunosuppression was reduced (azathioprine discontinued). Despite

these measures, no adequate decrease in CMV viral load was documented and the infection progressed to CMV tissue invasive disease with retinal and gastrointestinal involvement. After initiating systemic (50 mg per day, eGFR 20 ml/min) and intravitreal foscarnet therapy CMV VL decreased to undetectable levels after six weeks, retinal lesions improved and diarrhea stopped. A secondary prophylaxis with letermovir (240 mg qd) was initiated. Additionally, cytomegalovirus immune globulins were administered (despite missing clear evidence for benefit in this situation).¹

Three weeks after starting letermovir, the patient was diagnosed with an Epstein-Barr virus (EBV) positive gastrointestinal monomorphic post-transplant lymphoproliferative disorder (PTLD). From the time of PTLD diagnosis, cyclosporine A was first administered in reduced doses for a period of two weeks and then stopped completely without dose adjustment for letermovir. Immunosuppression was continued as prednisone monotherapy. After three weeks of accidentally insufficient letermovir dosing CMV VL increased (up to 10⁷7000 copies/mL) without clinical symptoms and foscarnet therapy was initiated. The previous inadequate low dosage of letermovir triggered CMV resistance testing and revealed combined CMV *UL97* (C607F) and a *UL54* mutations (N408D, foscarnet RI = 1.3).¹³ However, no genotypic letermovir resistance was discovered. After achieving complete viral suppression by foscarnet therapy, secondary CMV prophylaxis using letermovir was readministered (480 mg qd). However, one month after the restart of letermovir CMV VL peaked at 13²200 copies/mL and high level letermovir resistance (*UL56* C325Y, letermovir resistance index = >3000) was found.¹⁴ Treatment with foscarnet was restarted. The patient finally passed away because of treatment refractory PTLD.

DISCUSSION

Ganciclovir (GCV) resistant CMV is a common problem among SOT recipients who lack prior CMV immunity (CMV D+/R-). Risk factors include suboptimal dosing, intense immunosuppression, prolonged exposure to oral GCV, and mismatched CMV status (D+/R-).²² The usual incidence of resistance after ganciclovir therapy is 5% to 12%.²³⁻²⁵ Because of the good safety profile and the oral bioavailability letermovir may be considered as a valuable off-label option for secondary prophylaxis of GCV resistant CMV.

There is limited data on the use of letermovir as a secondary prophylaxis in SOT recipients (Table 1). Chong et al. successfully used letermovir as secondary prophylaxis in a heart transplant recipient with GCV resistant CMV.¹² In a recent case series, however, CMV breakthrough viremia in three out of eight lung transplant recipients with secondary letermovir prophylaxis was reported by Aryal et al.¹¹ Two patients with secondary prophylaxis failure received letermovir because of ganciclovir-induced leucopenia and one patient due to GCV resistant CMV. The emergence of genotypic letermovir resistance after failing the secondary prophylaxis was not reported in this case-series.

Failure of letermovir when administered as a secondary prophylaxis has also been reported in allogenic HSCT recipients. In a French cohort of 80 CMV seropositive allogenic HSCT recipients, four (5.5%) patients developed CMV breakthrough infection or disease after the initiation of letermovir.²⁶ In 3 out of 4 patients, further investigation of viral resistance showed CMV *UL56* C325Y/W mutations, both conferring high-level letermovir resistance.¹⁴ The *UL56* mutation C325Y was also detected by Jung et al. only a few weeks after initiating letermovir as secondary prophylaxis in an allogenic HSCT recipient.²⁷ Similar to the findings of *in vitro* studies, letermovir resistance mutation after failing secondary prophylaxis also primarily mapped at position *UL56* C325Y of the viral terminase complex in our two renal transplant patients and the HSCT recipients reported by Robin C et al.^{9,14,26,28} Mutations in the *UL89* or *UL51* genes, which have been described in *in vitro studies* as occasional localizations of letermovir resistance mutations were not detected in our two patients and not reported so far *in vivo* after failing secondary prophylaxis.²⁸ In addition, case series on letermovir used as salvage therapy

in SOT recipients also reported mixed results with a subset of patients developing letermovir resistance.^{10,29}

In SOT recipients it has been shown that CMV-specific immune monitoring might be beneficial for the identification of patients at high risk for development of late onset CMV disease (after discontinuation of antiviral prophylaxis).³⁰ Despite having robust CMV specific cell mediated immunity (CMI) case one was unable to control CMV. Most likely this was due to treatment of a rejection episode (high-dose corticosteroids) shortly after CMV-specific CMI measurement. We speculate that the inability to immunologically control CMV in case two was associated with the occurrence of PTLD and subsequent chemotherapy after the measurement of CMV-specific cell mediated immunity.

Letermovir resistance in our two renal transplant recipients emerged after a few months of secondary prophylaxis: In case one, letermovir resistance was detected after 106 days of prophylaxis, whereas in case two, the cumulative duration of prophylaxis before the onset of resistance was 98 days. In case two, however, there was a rise in CMV VL after 58 days of letermovir prophylaxis, although no genotypic letermovir resistance could be detected at that time. It might be possible that resistant subpopulations of virus at day 58 were present but not detectable by conventional Sanger sequencing.

In contrast to (val)ganciclovir, there are relevant drug-drug interactions with letermovir and immunosuppressive medication. When letermovir and cyclosporine are co-administered, a dose reduction (half dose) of letermovir is necessary. The mechanism by which cyclosporine increases letermovir serum concentration is likely due to the cyclosporine-mediated inhibition of OATP1B1/1B3 transporters, which are responsible for the hepatic uptake of letermovir (which is subsequently glucuronidated and biliary secreted).³¹ The relevance of the drug-drug interaction of cyclosporine and letermovir is nicely demonstrated in case two: The missed dose increase for letermovir after stopping cyclosporine led to a viral rebound. The intestinal PTLD which led to persistent diarrhea might have additionally impaired the absorption of letermovir in case two. Alain et al. showed that low blood concentration of letermovir may be responsible for CMV breakthrough viremia during both, primary and secondary prophylaxis, in allogenic HSCT recipients.³²

CONCLUSIONS

There is very limited experience with the use of letermovir as secondary prophylaxis for GCV resistant CMV. Strong immunosuppression, prolonged antiviral drug exposure or inadequate drug delivery are risk factors for developing GCV resistance.¹ These factors may remain unchanged when using letermovir as secondary CMV prophylaxis after the emergence of GCV resistance. Therefore, letermovir resistance may appear after a few weeks to months of prophylaxis. In view of lacking systematic data and clinical trials, we advocate for cautious use of letermovir as secondary prophylaxis for GCV resistant CMV in SOT recipients.

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FIGURE LEGENDS

Figure 1.

Timeline case one.

Timeline of medication, CMV viral load in plasma, resistance testing, and CMV-specific cell mediated immunity (measured by T-Track CMV®, expressed as spot forming cells per 2×10^5 input cells upon stimulation with pp65 or IE-1). Horizontal-axis indicates time from transplant (months). CMV, cytomegalovirus; DNA, deoxyribonucleic acid; VL, viral load, CMV IG, cytomegalovirus immunoglobulin (5000 units per dose); MMF, mycophenolate mofetil; SFC, spot forming cells; IE-1, immediate early-1 protein; pp65, phosphoprotein 65.

Figure 2.

Timeline case two.

Timeline of medication, CMV viral load in plasma, resistance testing, and CMV-specific cell mediated immunity (measured by T-Track CMV®, expressed as spot forming cells per 2×10^5 input cells upon stimulation with pp65 or IE-1). Horizontal-axis indicates time from transplant (months). CMV, cytomegalovirus; DNA, deoxyribonucleic acid; VL, viral load, CMV IG, cytomegalovirus immunoglobulin (3000 units per dose); MMF, mycophenolate mofetil; SFC, spot forming cells; IE-1, immediate early-1 protein; pp65, phosphoprotein 65.

TABLE 1 Clinical features of patients receiving letermovir as secondary prophylaxis after solid organ transplantation (Review of the literature)

Case	Type of transplant	Age	Sex	CMV status	Immunosuppression (at time of letermovir initiation)	Ganciclovir resistance	Reason for (val)ganciclovir discontinuation	Outcome	Letermovir Resistance testing	Year published
1	Single lung transplant	69	M	D+/R-	Tacrolimus, prednisone	No	Leucopenia	Successful	No	2019 (Aryal) ¹¹
2	Bilateral lung transplant	60	M	D+/R+	Cyclosporine, prednisone	No	Leucopenia	Successful	No	2019 (Aryal) ¹¹
3	Single lung transplant	68	F	D+/R-	Tacrolimus, prednisone, mycophenolate mofetil	No	Leucopenia	Successful	No	2019 (Aryal) ¹¹
4	Bilateral lung transplant	59	F	D+/R-	Tacrolimus, prednisone	No	Leucopenia	Failure	No	2019 (Aryal) ¹¹
5	Bilateral lung transplant	63	F	D+/R-	Tacrolimus, prednisone	No	Leucopenia	Failure	No	2019 (Aryal) ¹¹
6	Single lung transplant	67	F	D+/R-	Tacrolimus, prednisone	Yes (UL97, not specified)	Resistance	Failure	No	2019 (Aryal) ¹¹
7	Heart transplant	60	M	D+/R-	Tacrolimus, prednisone	Yes (UL97 C603W)	Resistance	Successful	No	2018 (Chong) ¹²

Abbreviations: CMV, Cytomegalovirus; D, Donor; F, Female; M, Male; R, Recipient; UL97; Ultra long 97.

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