Emergence and Persistence of Letermovir-Resistant Cytomegalovirus in a Patient With Primary Immunodeficiency

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Background. Letermovir is a novel cytomegalovirus antiviral that is approved for prophylaxis in hematopoietic stem cell transplantation recipients.

Methods. After obtaining informed consent, letermovir prophylaxis was started in a patient with a presumed late-onset primary, combined T- and B-cell immunodeficiency. Plasma CMV DNAemia was monitored with real-time polymerase chain reaction, and letermovir resistance analyses were performed using Sanger sequencing and Illumina MiSeq next-generation sequencing.

Results. A letermovir-resistant cytomegalovirus variant (C325Y mutation in UL56) emerged 17 weeks after start of prophylaxis. The letermovir-resistant variant was able to reactivate without drug selective pressure as this variant was again detected in plasma 20.6 weeks after stopping of letermovir.

Conclusions. This case indicates that the C325Y mutation in UL56 does not significantly alter fitness of cytomegalovirus in vivo.

Keywords. letermovir; resistance; viral fitness.

Human cytomegalovirus (CMV) causes significant complications in immunocompromised patients, which can be prevented and treated with the antiviral drugs (val)ganciclovir, foscarnet, and cidofovir. However, treatment is often complicated by drug toxicity, antiviral resistance, and the need for intravenous (i.v.) drug administration [1, 2]. Letermovir, a novel CMV antiviral that was recently approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for prophylactic use in hematopoietic stem cell transplantation (HSCT) recipients, has several advantages over currently used CMV antivirals. Letermovir can be administered either orally or intravenously and shows mild toxicity. In addition, there is no risk of cross-resistance with existing anti-CMV drugs due to a different mechanism of action [1, 3].

METHODS

After obtaining informed consent, letermovir prophylaxis was started in a patient with a presumed late-onset primary, combined T- and B-cell immunodeficiency. Plasma CMV-DNAemia was monitored by an internally controlled, in-house developed, dual target (UL54 and UL75), real-time polymerase chain reaction (PCR) using the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (code: 09/162, National Institute for Biological Standards and Control, Hertfordshire, Great Britain) as quantification standard. Cytomegalovirus-specific T-cell responses were analyzed with CMV ELISPOT (Oxford Immunotec Ltd., Abingdon, Great Britain) and CMV-QuantiFERON (QIAGEN GmbH, Hilden, Germany). Letermovir resistance analysis was performed by genotypic analysis of UL56 using Sanger sequencing and Illumina MiSeq (amplicon sequencing). Part of UL56 was amplified resulting in a PCR product, 1300-bp of which comprises the amino acids 231–369 described to be relevant for letermovir drug resistance. Amplification sensitivity was reached by performing a nested PCR including a first PCR with 25 cycles and a second PCR with 35 cycles. Ganciclovir, foscarnet, and cidofovir resistance analysis were performed by genotypic analysis of UL54 and UL97 using Sanger sequencing. All analyses were performed in ISO15189:2012 (or equivalent) accredited settings.

RESULTS

A 52-year-old female was referred to the Erasmus Medicial Center for analysis of a presumed primary immunodeficiency, after experiencing a recent episode of CMV colitis and CMV retinitis. Immunological analyses showed a combined B- and
T-cell deficiency with total T-cell numbers of 200 cells/µL (lower reference value [LRV], 700 cells/µL), CD4+ T cells of 40 cells/µL (LRV, 300 cells/µL), CD8+ T cells of 140 cells/µL (LRV, 200 cells/µL), total B cells of 70 cells/µL (LRV, 100 cells/µL), and a severe hypogammaglobulinemia (immunoglobulin [Ig]G 1.5 g/L [LRV, 7.0 g/L], IgA 0.28 g/L [LRV, 0.76 g/L], IgM 0.21 g/L [LRV, 0.45 g/L]). She was extensively evaluated for a secondary immunodeficiency, including bone marrow aspiration and total-body positron emission tomography/computed tomography scan, which not revealed an underlying (hematological) malignancy. She tested negative for human immunodeficiency virus (HIV). Gene panel testing comprising over 350 primary immunodeficiency-associated genes was performed using whole exome sequencing [4]. No known pathogenic mutations were found. Because no secondary cause of the immunodeficiency was found, a late-onset primary, combined T- and B-cell immunodeficiency was assumed, and she was started on monthly i.v. Ig replacement therapy and 480 mg of co-trimoxazole prophylaxis once daily.

Within the next 2 years, the patient required antiviral therapy for multiple episodes of CMV end-organ disease and CMV reactivations. Because the CMV colitis and CMV retinitis relapse was unresponsive to ganciclovir, a switch to foscarnet was required. Genotypic resistance analyses of plasma and colonic biopsies showed the M460I mutation in UL97, which confers a 4-fold reduced susceptibility to ganciclovir [5]. During the next 18 months, the patient received a total of 10 months i.v. foscarnet for relapses of CMV colitis and retinitis and as preemptive therapy (Figure 1). To stimulate her T cells, interferon alpha-2a therapy was started, but it was halted after 3 months because of inefficacy. Cytomegalovirus IgGs were combined with foscarnet to treat relapses of CMV end-organ disease. After these 18 months, no specific T-cell responses against CMV were detected (not reactive CMV-ELISPOT and CMV-QuantiFERON), indicating a persisting high risk for severe active CMV infections. Foscarnet i.v. treatments required hospitalization and were complicated by anemia, leukopenia, hypomagnesemia, and renal impairment. Therefore, we explored other treatment options. Cidofovir has considerable renal toxicity and cidofovir resistance mutations and can confer cross-resistance to foscarnet. Maribavir and brincidofovir are not approved by the FDA or EMA yet. Letermovir is FDA and EMA approved, has a mild toxicity profile, and does not confer cross-resistance to other CMV antivirals. Therefore, after obtaining informed consent, the patient was switched to off-label letermovir prophylaxis (480 mg orally once daily) as soon as plasma CMV DNAemia was <50 IU/mL (limit of quantification). During letermovir prophylaxis, several breakthroughs of CMV replication occurred (Figure 1). The patient did not experience CMV end-organ disease during or after letermovir prophylaxis failure. The first breakthrough coincided with an episode of vomiting and may have resulted in poor letermovir uptake. No causes could be identified for

![Figure 1](https://academic.oup.com/ofid/article-abstract/6/9/ofz375/5554080/5554080)

**Figure 1.** Course of plasma cytomegalovirus (CMV) DNAemia during treatment with intravenous foscarnet (black bars) and oral letermovir (gray bar). Dotted horizontal line at 1.7 log_{10} is the lower limit of quantification (50 IU/mL). Boxes with gray arrows show the results from genotypic resistance analyses.
of higher dosages for off-label treatment of 4 transplant patients
the 480 mg per day dosage [8]. Turner et al [9] reported the use
1440 mg per day for up to 14 days with similar adverse events as
receiving letermovir dosages ranging from 720 mg per day up to
higher dose of combination therapies or cycling of therapies.
However, it should be noted that without immune control, any
antiviral therapy of CMV will eventually fail and acquired an-
tiviral drug resistance will emerge [14]. More data are needed
to provide insight into in vivo-detected mutations, interpretation
of genotyping results, and the clinical relevance of the results.

DISCUSSION
This is the first report on the off-label use of letermovir in a
patient with presumed primary, combined immunodeficiency.
Letermovir was well tolerated, but several breakthroughs of
CMV replication occurred, with emerging antiviral resistance.
Our patient was treated with a dose of 480 mg per day, which
has proven to be safe and effective as CMV prophylaxis among
HSCT recipients in a phase 3 clinical trial [3]. Nevertheless,
7.7% of the participants experienced a breakthrough of CMV
replication during prophylaxis. Higher dosages could be more
effective in preventing these breakthroughs, but data on this
topic are sparse. The registration label reports healthy subjects
receiving letermovir dosages ranging from 720 mg per day up to
1440 mg per day for up to 14 days with similar adverse events as
the 480 mg per day dosage [8]. Turner et al [9] reported the use
of higher dosages for off-label treatment of 4 transplant patients
with CMV retinitis (720 and 960 mg per day), but resistance
emerged in 2. It should be noted that there is a lack of safety data
on sustained high doses of letermovir (>480 mg per day) in pub-
lished literature. Currently, there is an urgent need for additional
data on dosage and effectiveness in different patient groups.

There are an increasing number of reports of acquired
ermovir resistance. Among HSCT recipients, UL56 mutations
V236M [1, 3], C325W [3], C325F [10], and C325Y [11, 12] are
reported; in solid organ transplants patients, the C325Y [9, 13]
and C325F [9] are reported. In this case, the UL56 mutation
t lasted for 2 weeks of letermovir prophylaxis. Taken
together, these data indicate that UL56 mutation at position C325
is the main acquired letermovir resistance mutation in vivo. We
recommend tight monitoring of CMV DNAemia during pro-
phylaxis with letermovir because timely resistance might occur.
Only a single transition can result in the C325Y mutation re-
ducing susceptibility by >3000-fold with minimal impact on viral
replication in vitro [6, 7]. Our results indicate that CMV strains
harboring the C325Y mutation in UL56 are able to reactivate in
humans without selective drug pressure because the C325Y mu-
tation was still detected in plasma during the second episode of
CMV reactivation occurring 20.6 weeks after stopping letermovir.
It is interesting to note that during the first episode of CMV reac-
tivation, the UL56 C325Y mutation was not detected (9.6 weeks
after stopping letermovir), although 1 week later the mutation
was detected. In between these 2 samples, foscarnet was started.
Although we cannot rule out a decreased foscarnet susceptibility
for the variant harboring the UL56 C325Y mutation, it seems
unlikely as previous in vitro studies showed no cross-resistance
between letermovir and foscarnet, no foscarnet mutations were
detected in this particular variant and the patient responded well
to foscarnet treatment. We hypothesize that during this episode
multiple CMV variants were reactivating and that the letermovir-
resistant variant was the majority in the second sample.

CONCLUSIONS
Regardless of the apparent low barrier to resistance, letermovir
can positively contribute to management of CMV infections.
First, letermovir has a favorable safety profile compared with
other CMV antivirals and is not associated with myelotoxicity
and nephrotoxicity. Second, letermovir can be used orally so no
hospitalization is needed nor an i.v. access. Finally, letermovir
targets the CMV terminase complex instead of CMV deoxyribo-
nucleic acid polymerase so no cross-resistance with the other ap-
proved CMV antivirals occurs [3], suggesting discussions about
administration of combination therapies or cycling of therapies.
However, it should be noted that without immune control, any
antiviral therapy of CMV will eventually fail and acquired an-
tiviral drug resistance will emerge [14]. More data are needed
to provide insight into in vivo-detected mutations, interpretation
of genotyping results, and the clinical relevance of the results.
This can all be provided by a CMV registry. Moreover, generated knowledge can contribute to CMV consensus guidelines and treatment algorithms. Databases established as resistance surveillance and resistance interpretation for HIV and hepatitis C virus are successful and provide useful information. A similar approach for CMV would be highly recommended.

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References