



Case Report

In Vivo Emergence of UL56 C325Y Cytomegalovirus Resistance to Letermovir in a Patient with Acute Myeloid Leukemia after Hematopoietic Cell Transplantation

Jochen J Frietsch^{1*}, Detlef Michel^{2*}, Thomas Stamminger², Friederike Hunstig¹, Sebastian Birndt¹, Ulf Schnetzke¹, Sebastian Scholl¹, Andreas Hochhaus¹ and Inken Hilgendorf¹.

* Both authors contributed equally.

¹ Klinik für Innere Medizin II, Hämatologie und internistische Onkologie, Universitätsklinikum Jena, Jena, Germany.

² Institut für Virologie, Universitätsklinikum Ulm, Ulm, Germany.

Competing interests: The authors have declared that no competing interests exist.

Abstract. CMV associated tissue-invasive disease is associated with a considerable risk of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Recently, the terminase inhibitor letermovir (LMV) has been approved for prophylaxis of CMV infection in HSCT. We hereby report a 60-year-old female experiencing CMV reactivation after HSCT in a CMV seronegative donor-constellation. Due to ongoing elevated CMV viral load and drug-associated myelosuppression, which prevented ganciclovir therapy, treatment was replaced by foscarnet. Due to nephrotoxicity, foscarnet was switched to LMV. The patient developed skin GvHD and prednisolone was started. Subsequently, CMV viremia worsened despite LMV therapy. Genotyping revealed the mutation C325Y of the CMV UL56 terminase being associated with high-level resistance against LMV. Prolonged uncontrolled low-level viremia due to prednisolone treatment may have favored the selection of drug-resistant CMV. Despite the excellent toxicity profile of LMV, physicians should be aware of risk factors for the emergence of resistance.

Keywords: Cytomegalovirus; Disease; Hematopoietic stem cell transplantation; Reactivation; Letermovir.

Citation: Frietsch J.J., Michel D., Stamminger T., Hunstig F., Birndt S., Schnetzke U., Scholl S., Hochhaus A., Hilgendorf I. Resistance of cytomegalovirus towards letermovir due to C325Y mutation in UL56 in a patient after hematopoietic stem cell transplantation. *Mediterr J Hematol Infect Dis* 2019, 11(1): e2019001, DOI: <http://dx.doi.org/10.4084/MJHID.2019.001>

Published: January 1, 2019

Received: September 21, 2018

Accepted: November 14, 2018

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Correspondence to: Dr. med. Jochen Frietsch. Universitätsklinikum Jena, Abteilung Hämatologie und Internistische Onkologie Am Klinikum 1, 07747 Jena, Germany. E.mail: jochen.frietsch@med.uni-jena.de. ORCID ID <https://orcid.org/0000-0002-1476-8059>

Introduction. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for a huge variety of malignant and even non-malignant diseases.¹ Viral infections and reactivations, especially of cytomegalovirus (CMV), and its associated tissue-invasive disease, remain a serious complication following HSCT.² For a long time, the antiviral drugs ganciclovir (GCV)/valganciclovir, foscarnet (FOS), cidofovir and acyclovir/valacyclovir have been used for prophylaxis

or pre-emptive therapy but were limited by side effects and/or the selection of viral mutations that confer antiviral drug resistance. Recently the armamentarium has been widened by the administration of maribavir, brincidofovir, and letermovir (LMV). Targeting the subunit UL56 of the terminase enzyme complex, LMV specifically inhibits the cleavage and packaging of newly synthesized viral DNA.³ A recent phase III trial demonstrated that LMV prophylaxis after allogeneic HSCT resulted in a significantly lower risk of active

CMV infection compared to placebo.⁴ In addition, LMV seems to be well tolerated without the risk of myelo- or nephrotoxicity. However, experimental in vitro data suggested that LMV may possess a low genetic barrier to resistance.⁵ Here we report the case of a patient after allogeneic HSCT with prolonged CMV viremia with a C325Y mutation-based resistance to LMV, being selected in vivo.

Case Presentation. We report a 60-year-old, CMV seropositive female patient with acute myeloid leukemia (AML). Cytogenetics revealed trisomy 8 in 3 out of 10 metaphases. Verification of mutations in ASXL1, EZH2 and NPM1 led to high risk classification according to European LeukemiaNet ELN guidelines.⁶ Administering induction and consolidation chemotherapy (cytarabine 1.000 mg/m², bid, on day 1, 3, 5 and 7, idarubicin 12 mg/m², qd, on days 1-3) resulted in achieving complete remission. After conditioning therapy with treosulfan (day -6 to -4 at doses of 14 g/m², qd), fludarabine (day -6 to -2 at doses of 30 mg/m², qd) and anti-thymocyte globulin (Grafalon[®]; neovii Biotech, days -4 to -2 at a dose of 20 mg/kg body weight (bwt)) the patient received 6.06 x 10⁶ CD34+ peripheral blood stem cells/kg bwt from a 24-year-old, unrelated, male, human leukocyte antigen allele mismatched 9/10, EBV seropositive, CMV seronegative donor. Prophylaxis of Graft-versus-host disease (GvHD) consisted of cyclosporine (CsA), starting on day -1, combined with methotrexate (MTX). CsA was maintained at therapeutic plasma levels. Acyclovir was administered as antiviral prophylaxis.

Loads of CMV were routinely monitored once a week by PCR technique using whole-blood (for detailed information about viral copy numbers see **figure 1**). CMV reactivation with 7 x 10⁴ copies/ml was detected on day +43. Despite a change in antiviral medication to GCV at a dosage of 5 mg/kg bwt, bid, viral load kept on increasing up to 4.8 x 10⁵ copies/ml after ten days of initiation of treatment. The viral load finally decreased underuse of FOS at a dosage of 90 mg/kg bwt bid and early but slow reduction of immunosuppressive therapy. Additionally, we administered 1 ml/kg bwt CMV Immunoglobulins (Cytotect[®] CP Biotest) on day +48. The CMV-treatment schedule is given in **figure 1**.

Due to delayed engraftment, several bone marrow aspirates were obtained, revealing increasing chimerism from 89% on day +30, 94% on day +54 up to 100% since day +82. As a result of minimal residual disease (MRD) of AML, we quickly reduced CsA. This resulted in decreasing leukemia associated molecular markers. However, CMV copy numbers raised from 1.5-3.5 x 10³ copies/ml up to 3.9 x 10⁵ copies/ml (please refer to **figure 1**, day 88 et seq.) and EBV reactivation (up to 0.83-1.65 x 10⁵) despite sustained

administration of FOS. Except for nephrotoxicity, no clinical side effects of FOS occurred. EBV reactivation was effectively treated with the monoclonal CD20 antibody rituximab throughout four weeks, at a dosage of 375 mg/m² per week (**Figure 1**).⁷

At the same time, CMV copy number increased despite the continuation of treatment with FOS. Therefore, we attempted to exclude the existence of viral mutations by DNA sequencing following nested PCR amplification. With modifications, amplification and sequencing of UL56 were performed as described previously.⁸ The method allows identification of the UL56 coding region from amino acids 1 to 620. Albeit clinically expected, verifying the viral kinase UL97 and the viral polymerase UL54 as wild-type, no mutation conveying resistance was demonstrable. A retrospective analysis revealed no mutation in the viral terminase region UL56, too. As a consequence, administration of CsA was terminated at day +118.

Based on delayed engraftment, drug-associated myelotoxicity and nephrotoxicity, and prolonged hospitalization, we initiated LMV at a dose of 480 mg qd. The patient was discharged from stationary treatment, and LMV resulted in an increase first, and within a treatment period of five weeks in an impressive decrease of CMV copy numbers (from 90.000 at LMV initiation up to 1.000.000 to 8.200 per milliliter) as already reported for other cases.⁹ Due to the occurrence of herpes stomatitis, acyclovir was administered, adapted to renal function. Simultaneously, the patient developed a maculopapular rash on day +155 affecting the lower arms and the abdominal skin. Subsequently, a skin biopsy was performed, and suspected acute GvHD confirmed by histology.

Consequently, prednisolone 25 mg qd was started at day +165 in the absence of any signs of gastrointestinal or liver involvement of GvHD and tapered without recurrence of acute GvHD afterward. However, under the intensified immunosuppression the viral load increased up to 410.000 copies per milliliter despite the continuation of LMV treatment. Upon genotyping, mutation C325Y (cytosine at amino acid position 325 was substituted by tyrosine) was detected within UL56 which is supposed to confer high-level resistance to LMV.¹⁰

Consequently, administration of LMV was stopped, and FOS application, adapted to renal function, 3.000 mg bid commenced once again in combination with administration of CMV-hyperimmune globulin. This change in antiviral treatment resulted in a decrease of viral loads. On day +292, the patient is alive in complete remission of AML without signs of GvHD or clinical signs of active CMV infection, still receiving FOS without any side effects. Of note, during the whole course of treatment, CMV was below 1 x 10³ copies/ml only until day +42, between day +63 and

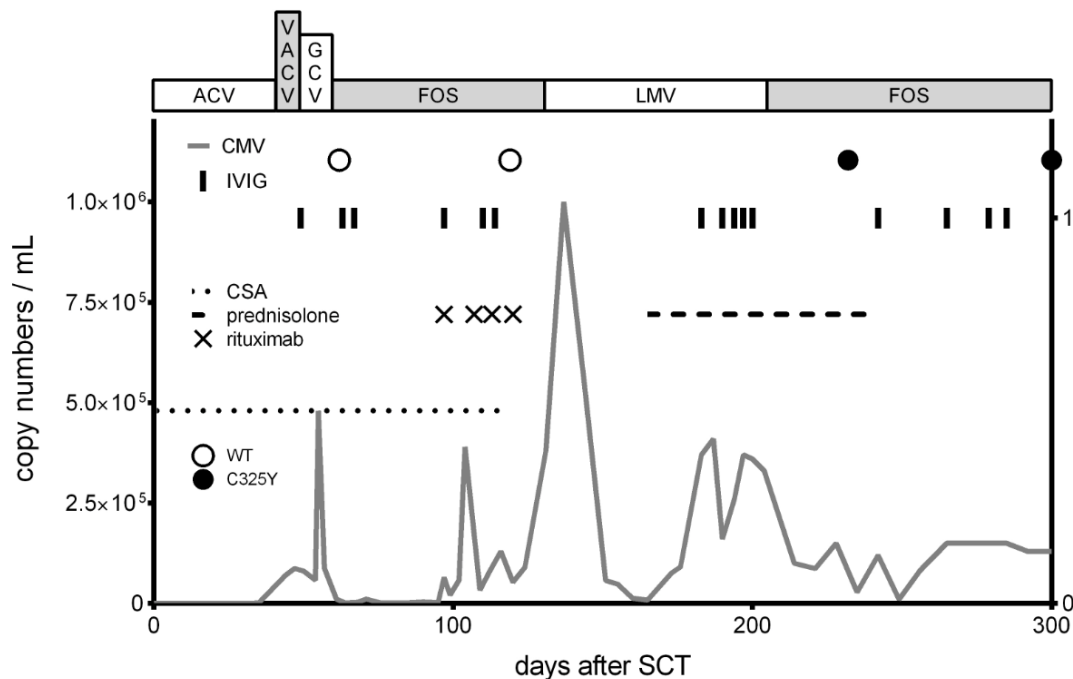


Figure 1. Clinical course of the patient. Cytomegalovirus (CMV) copy numbers in folds of 10^5 in gray shown as drawn through line; administration of hyperimmune globulines (IVIG), cyclosporine (CsA), prednisolone, rituximab (depicted by crosses) and antiviral medication (ACV: acyclovir; FOS: foscarnet; GCV: ganciclovir; LMV: letermovir; VACV: valacyclovir) as indicated above, detection of UL56 wild type (WT) is depicted as empty circle and UL56 C325Y as black circle respectively.

+67 as well as day +75 and +84 after HSCT.

Discussion and Conclusion. CMV associated tissue-invasive disease (e.g., pneumonitis, retinitis) is known to cause significant morbidity and mortality in patients after allogeneic HSCT.² However, CMV replication may also exert anti-leukemic effects after HSCT in AML patients. Based on that conflict, physicians have to choose appropriate antiviral strategies.¹¹

Side effects like nephrotoxicity, electrolyte disturbances, and myelotoxicity sometimes restrict the treatment with distinct antiviral drugs. Concerning its safety profile, the newly approved drug LMV appears to be superior to other anti-cytomegaloviral substances. However, since LMV specifically interferes with cleavage and packaging of viral DNA, without affecting viral DNA replication, this may result in prolonged detection of CMV DNA after the initiation of LMV therapy.⁵ Furthermore, the case presented in this study underlines that LMV is highly specific for CMV without an inhibitory effect on related herpesviruses such as HSV or VZV. Consequently, concomitant prophylaxis with acyclovir is compulsory in order to prevent disease due to HSV/VZV reactivation as observed by the HSV-associated stomatitis in our patient.

Experimental *in vitro* data suggested an early selection of cytomegaloviruses with resistance-associated mutations in the presence of LMV. Thus, it was proposed that CMV may exhibit a low genetic barrier towards LMV resistance development necessitating continuous surveillance during

treatment.¹² So far, the UL56 V236M mutation has been selected *in vivo* during two clinical trials.^{5,8} The authors of a subsequent phase III trial stated that the development of breakthrough CMV viremia with confirmed UL56 mutations had been observed.^{4,13} DNA sequence analysis of the UL56 and UL89 coding regions was performed on samples obtained from 28 letermovir-treated patients who had received at least one dose of study drug and experienced prophylaxis failure. Two patients were identified as having a letermovir-resistance substitution, pUL56 V236M or C325W. These substitutions were identified from on-treatment samples (www.accessdata.fda.gov, Reference ID 4179078, ClinicalTrials.gov Identifier: NCT02137772).¹⁴ Here, we report for the first time *in vivo* cytomegalovirus carrying the UL56 mutation C325Y, which was detected by CMV genotyping upon rapidly increasing viral loads in a patient under LMV treatment. *In vitro* data indicate that this mutation is associated with high-grade LMV resistance increasing the 50% effective concentration of LMV >5.000-fold.^{12,13} In line with the published risk factors by El Chaer et al.,¹⁵ it is tempting to suggest, that prolonged uncontrolled low-level CMV viremia might have favored the emergence of letermovir resistance.

In the future, the combination of antiviral drugs with different mechanisms of action may be used synergistically to reduce the incidence of mutations and side effects. In addition, transfer of *ex vivo*-generated CMV-specific T-cells can suppress CMV-reactivation by re-establishing functional antiviral immune responses in immunocompromised hosts.¹⁶

References:

1. Mohty B, Mohty M. Long-term complications and side effects after allogeneic hematopoietic stem cell transplantation: an update. *Blood Cancer J*. 2011 Apr;1(4):e16. <https://doi.org/10.1038/bcj.2011.14>
2. Gandhi MK, Khanna R. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. *Lancet Infect Dis*. 2004;4(12):725-38. [https://doi.org/10.1016/S1473-3099\(04\)01202-2](https://doi.org/10.1016/S1473-3099(04)01202-2)
3. Frange P, Leruez-Ville M. Maribavir, brincidofovir and letermovir: Efficacy and safety of new antiviral drugs for treating cytomegalovirus infections. *Med Mal Infect*. 2018. <https://doi.org/10.1016/j.medmal.2018.03.006> PMID:29650261
4. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF, et al. Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation. *N Engl J Med*. 2017;377(25):2433-44. <https://doi.org/10.1056/NEJMoa1706640> PMID:29211658
5. Razonable RR. Role of letermovir for prevention of cytomegalovirus infection after allogeneic haematopoietic stem cell transplantation. *Curr Opin Infect Dis*. 2018. <https://doi.org/10.1097/QCO.0000000000000459> PMID:29746444
6. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-47. <https://doi.org/10.1182/blood-2016-08-733196> PMID:27895058 PMCid:PMC5291965
7. Styczynski J, van der Velden W, Fox CP, Engelhard D, de la Camara R, Cordonnier C, et al. Management of Epstein-Barr Virus infections and post-transplant lymphoproliferative disorders in patients after allogeneic hematopoietic stem cell transplantation: Sixth European Conference on Infections in Leukemia (ECIL-6) guidelines. *Haematologica*. 2016;101(7):803-11. <https://doi.org/10.3324/haematol.2016.144428> PMID:27365460 PMCid:PMC5004459
8. Lischka P, Michel D, Zimmermann H. Characterization of Cytomegalovirus Breakthrough Events in a Phase 2 Prophylaxis Trial of Letermovir (AIC246, MK 8228). *J Infect Dis*. 2016;213(1):23-30. <https://doi.org/10.1093/infdis/jiv352> PMID:26113373
9. Stoelben S, Arns W, Renders L, Hummel J, Muhlfeld A, Stangl M, et al. Preemptive treatment of Cytomegalovirus infection in kidney transplant recipients with letermovir: results of a Phase 2a study. *Transpl Int*. 2014;27(1):77-86. <https://doi.org/10.1111/tri.12225> PMID:24164420
10. Ligat G, Cazal R, Hantz S, Alain S. The human cytomegalovirus terminase complex as an antiviral target: a close-up view. *FEMS Microbiol Rev*. 2018;42(2):137-45. <https://doi.org/10.1093/femsre/fuy004> PMID:29361041 PMCid:PMC5972660
11. Elmaagacli AH, Koldehoff M. Cytomegalovirus replication reduces the relapse incidence in patients with acute myeloid leukemia. *Blood*. 2016;128(3):456-9. <https://doi.org/10.1182/blood-2016-04-713644> PMID:27216219
12. Chou S. Rapid In Vitro Evolution of Human Cytomegalovirus UL56 Mutations That Confer Letermovir Resistance. *Antimicrob Agents Chemother*. 2015;59(10):6588-93. <https://doi.org/10.1128/AAC.01623-15> PMID:26259791 PMCid:PMC4576131
13. Goldner T, Hempel C, Ruebsamen-Schaeff H, Zimmermann H, Lischka P. Geno- and phenotypic characterization of human cytomegalovirus mutants selected in vitro after letermovir (AIC246) exposure. *Antimicrob Agents Chemother*. 2014;58(1):610-3. <https://doi.org/10.1128/AAC.01794-13> PMID:24189264 PMCid:PMC3910730
14. Letermovir (MK-8228) Versus Placebo in the Prevention of Clinically-Significant Cytomegalovirus (CMV) Infection in Adult, CMV-Seropositive Allogeneic Hematopoietic Stem Cell Transplant Recipients (MK-8228-001) [Available from: <https://ClinicalTrials.gov/show/NCT02137772>
15. El Chaer F, Shah DP, Chemaly RF. How I treat resistant cytomegalovirus infection in hematopoietic cell transplantation recipients. *Blood*. 2016. <http://www.bloodjournal.org/content/bloodjournal/early/2016/10/19/blood-2016-06-688432.full.pdf>
16. Gary R, Aigner M, Moi S, Schaffer S, Gottmann A, Maas S, et al. Clinical-grade generation of peptide-stimulated CMV/EBV-specific T cells from G-CSF mobilized stem cell grafts. *J Transl Med*. 2018;16(1):124. <https://doi.org/10.1186/s12967-018-1498-3> PMID:29743075 PMCid:PMC5941463