Letermovir Resistance in Hematopoietic Stem Cell Transplant Recipients: The Risks Associated with Cytomegalovirus Prophylaxis

Scott H. James

Department of Pediatrics, Division of Infectious Diseases, The University of Alabama at Birmingham, Birmingham, Alabama

(See the Major Article by Douglas et al, on pages 1117–26.)

Keywords. Cytomegalovirus; letermovir; antiviral resistance.

Cytomegalovirus (CMV) remains a major cause of disease and death in allogeneic hematopoietic stem cell transplant (HSCT) recipients. In CMV-seropositive patients, early CMV reactivation is particularly associated with poor outcomes, including lower overall survival rates [1]. The use of antiviral prophylaxis for the prevention of CMV reactivation and subsequent reduction in clinically significant CMV infection in high-risk HSCT populations has been limited by the high frequency of drug-associated toxic effects, including clinically significant myelosuppression, associated with agents such as ganciclovir and valganciclovir. Instead, clinicians have often relied on preemptive therapy strategies aimed at early detection of CMV reactivation in blood and initiation of antiviral therapy prior to signs of clinical disease [2]. Preemptive therapy has the benefit of reducing exposure to drug toxic effects, but permitting early CMV viremia—even at low copy numbers—is associated with an increased risk of overall mortality within the first year of HSCT,

even in patients in whom preemptive therapy has been appropriately initiated [3]. This emphasizes the need for a safe and effective antiviral agent that could be tolerated as CMV prophylaxis for HSCT recipients in the posttransplantation period.

Letermovir (LET) was identified as a candidate to meet this need for reliable CMV prophylaxis in HSCT recipients. LET is a nonnucleoside agent that inhibits the terminal phase of CMV replication by targeting the CMV terminase complex, comprised of viral protein subunits pUL56, pUL89, and pUL51 [4]. Along with the portal protein pUL104, the terminase complex acts to cleave multimeric DNA concatemers into single-unit genomes, packaging them into preformed viral capsids before cellular egress. LET interferes with the binding of viral DNA concatemers to the CMV terminase complex, thereby inhibiting viral replication. LET is highly bioavailable, allowing for both oral and intravenous formulations. Notably, LET is not associated with myelosuppression and its mechanism of action is distinct from that of the other currently approved anti-CMV agents, all of which act as inhibitors of viral DNA polymerase. This novel mechanism of action makes LET an especially attractive agent in immunocompromised populations because it is less likely to induce cross-resistance to polymerase inhibitors [5].

LET gained Food and Drug Administration approval for CMV prophylaxis in seropositive adult HSCT recipients in 2017. The pivotal trial, conducted by Marty et al [6], was a phase 3, double-blind, placebo-controlled investigation of LET prophylaxis given through week 14 after transplantation in CMV-seropositive patients with undetectable viral loads at the time of randomization. Compared with placebo, LET prophylaxis in this population was associated with a reduction in clinically significant CMV infection ($P < .001$), as well as lower all-cause mortality rate ($P = .03$), at 24 weeks. LET was well tolerated and no safety concerns were identified, including no significant myelosuppression. It is worth noting that an uptick in clinically significant CMV infection was observed beginning about 4 weeks after discontinuation of LET prophylaxis, the occurrence of which indicates that in certain high-risk clinical scenarios a longer duration of prophylactic therapy might be beneficial. (A phase 3 clinical trial investigating LET prophylaxis for 200 days after HSCT is currently underway [clinicaltrials.gov identifier NCT03930615].)

The authors of the 2017 study noted, however, that CMV-seropositive patients receiving LET prophylaxis can experience reactivation with breakthrough viremia and the development of reduced susceptibility to LET [6]. Indeed,

Received 30 October 2019; editorial decision 30 October 2019; accepted 31 October 2019; published online November 29, 2019.

Correspondence: Scott H. James, 1600 Seventh Ave S, CHB 308, Birmingham, AL 35233 (sjames@peds.uab.edu).

The Journal of Infectious Diseases® 2020;221:1036–8
© The Author(s) 2019. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com. DOI: 10.1093/infdis/jiz578

a limited resistance analysis reported along with the results of the trial identified a resistance-associated variant (RAV) encoding pUL56 V236M in 1 patient, a concerning finding given that the likelihood of a low threshold for LET resistance had already been raised during the course of preclinical investigation [6].

In vitro selection studies have suggested a low genetic barrier to the development of LET resistance, with selection of LET-resistant CMV strains achievable at relatively low passages in error-prone exonuclease mutant CMV strains, as well as in baseline CMV laboratory strains [7–10]. These studies included phenotypic characterization of recombinant viruses, demonstrating that though most LET RAVs map to *UL56*, CMV is capable of numerous genetic pathways to LET resistance (including *UL89* and *UL51*). This suggests a multiplier effect in which certain combinations of mutations potentiate a reduction in LET susceptibility, and it also suggests that many LET RAVs are associated with a low fitness cost. In vitro data are not necessarily directly applicable to clinical experience, but, taken together, these findings highlight the need for rigorous resistance analyses to be included in all clinical trials involving LET.

In this issue of *The Journal of Infectious Diseases*, Douglas and colleagues [11] provide an example of the type of resistance analysis that will be crucial to a proper understanding of the role of LET in preventing and treating CMV disease in immunocompromised patients. Working from the phase 3 trial [6], the study team performed amplicon-based next-generation sequencing of the CMV genes *UL56* and *UL89* in patients who received 14 weeks of LET prophylaxis or placebo and in whom clinically significant CMV infection developed through week 24 after HSCT.

Douglas et al report a similar overall incidence of genotypic variants among patients who received LET and those

who received placebo (inclusive of natural polymorphisms with no significant impact on antiviral susceptibility). Of 79 patients who received LET and in whom clinically significant CMV infection developed, *UL56* genotyping was achieved on 50 patients, in whom 4 RAVs were identified among 3 patients. Known RAVs encoding pUL56 V236M and C325W were identified at 99% in 1 patient each, with both arising during the course of LET prophylaxis (on days 62 and 39, respectively).

Two novel variants encoding pUL56 E237G and R369T were identified and were subsequently demonstrated to confer resistance to LET via recombinant phenotyping. E237G occurred in 1 patient at a low level (4%) on study day 42, which was 32 days after LET had been discontinued because of the onset of clinically significant CMV infection. R369T was detected at 3 different time points in the same patient in whom C325W was also found. No *UL89* RAVs were identified, nor was any resistance detected in the placebo group. Overall, this constitutes a low incidence of LET resistance detected in this population of HSCT recipients who developed clinically significant CMV infection while on 14 weeks of LET prophylaxis.

This study's analysis benefitted from a quality control measure in the form of repeat replicate testing of novel variants to distinguish true genotypic variants from polymerase chain reaction-generated artifacts. This served to reduce the number of false-positive findings, which is a particular risk when detecting minority subpopulation variants in specimens with low viral loads near the limit of detection.

One interesting finding, the significance of which is still to be determined, involves the recombinant phenotyping of the novel *UL56* variant, E237G. A CMV recombinant strain with the E237G substitution yielded a 13-fold decrease in susceptibility to LET compared with wild-type CMV, but it was also determined to have a modest 2.1-fold decrease

in susceptibility to ganciclovir. Although this does not constitute a high level of ganciclovir resistance, it is statistically significant and runs contrary to the assertion that LET RAVs are not associated with cross-resistance. It has been shown that certain combinations of *UL54* and *UL56* mutations in the setting of sequential therapy with LET and a polymerase inhibitor could yield broad cross-resistance [10], but Douglas et al do not indicate that the patient in question received ganciclovir or valganciclovir before study day 42. Valganciclovir treatment was started on that same day, however (presumably after specimen collection). It is also noted that E237G occurred in low abundance (4% of the next-generation sequencing reads) in a specimen with low CMV copy number (<151 copies/mL). The significance of the E237G cross-resistance phenotype is uncertain.

Although it is reassuring that LET resistance was uncommon in this phase 3 trial of LET prophylaxis in HSCT recipients, it would be prudent to discriminate the differing levels of risk associated with CMV prophylaxis and CMV treatment. Douglas et al are correct to point out that their findings do not directly translate to LET treatment situations. In the setting of end-organ disease and higher viral burden, the low genetic barrier to the development of LET resistance may well become a significant limitation [12, 13]. More data are needed, and a phase 2 clinical trial evaluating the safety and efficacy of LET treatment in immunocompromised patients with refractory or resistant CMV infection or disease is underway (clinicaltrials.gov identifier NCT03728426). As these studies progress, it will be important to carry out well-thought-out resistance analyses, such as Douglas and colleagues have conducted. In the meantime, clinicians should maintain a low threshold for genotypic resistance testing when using LET in the setting of possible CMV infection or disease.

Potential conflicts of interest. Author certifies no potential conflicts of interest.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Teira P, Battiwalla M, Ramanathan M, **et al.** Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. *Blood* **2016**; 127:2427–38.
2. Rubin RH. Preemptive therapy in immunocompromised hosts. *N Engl J Med* **1991**; 324:1057–9.
3. Green ML, Leisenring W, Xie H, **et al.** Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study. *Lancet Haematol* **2016**; 3:e119–27.
4. Gentry BG, Bogner E, Drach JC. Targeting the terminase: an important step forward in the treatment and prophylaxis of human cytomegalovirus infections. *Antiviral Res* **2019**; 161:116–24.
5. 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**; 58:610–3.
6. Marty FM, Ljungman P, Chemaly RF, **et al.** Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med* **2017**; 377:2433–44.
7. Chou S. Rapid in vitro evolution of human cytomegalovirus UL56 mutations that confer letermovir resistance. *Antimicrob Agents Chemother* **2015**; 59:6588–93.
8. Chou S, Satterwhite LE, Ercolani RJ. New locus of drug resistance in the human cytomegalovirus UL56 gene revealed by in vitro exposure to letermovir and ganciclovir. *Antimicrob Agents Chemother* **2018**; 62:e00922–18.
9. Chou S. A third component of the human cytomegalovirus terminase complex is involved in letermovir resistance. *Antiviral Res* **2017**; 148:1–4.
10. Piret J, Goyette N, Boivin G. Drug susceptibility and replicative capacity of multidrug-resistant recombinant human cytomegalovirus harboring mutations in *UL56* and *UL54* genes. *Antimicrob Agents Chemother* **2017**; 61:e01044–17.
11. Douglas CM, Barnard R, Holder D, **et al.** Letermovir resistance analysis in a clinical trial of cytomegalovirus prophylaxis for hematopoietic stem cell transplant recipients. *J Infect Dis* **2019**; 11:e2019001.
12. Frietsch JJ, Michel D, Stamminger T, **et al.** In vivo emergence of UL56 C325Y cytomegalovirus resistance to letermovir in a patient with acute myeloid leukemia after hematopoietic cell transplantation. *Mediterr J Hematol Infect Dis* **2019**; 11:e2019001.
13. Cherrier L, Nasar A, Goodlet KJ, Nailor MD, Tokman S, Chou S. Emergence of letermovir resistance in a lung transplant recipient with ganciclovir-resistant cytomegalovirus infection. *Am J Transplant* **2018**; 18:3060–4.