Mediterranean Journal of Hematology and Infectious Diseases

Review Article

Update on Cytomegalovirus Infection Management in Allogeneic Hematopoietic Stem Cell Transplant Recipients. A Consensus Document of the Spanish Group for Hematopoietic Transplantation and Cell Therapy (GETH-TC)

Supplementary Material

Table 1: Grading system for the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommendations[1].
Recommendation Grade

- Grade A: Strongly supports the recommendation.
- Grade B: Moderately supports the recommendation.
- Grade C: Marginally supports the recommendation.
- Grade D: Against the recommendation.

Quality of evidence

- Level I: evidence from at least one properly designed, randomized, controlled clinical trial (CT).
- Level II*: evidence from at least one well-designed CT (without randomization); from analytical cohort or case-controlled studies (preferably from more than one center); from multiple time series; or significant results in uncontrolled studies.
- Level III: Evidence from expert opinion, based on clinical experience, descriptive case studies, or expert committee reports.

*Added index for level II quality of evidence
• r: meta-analysis or systematic review of randomized controlled trials
• t: transferred evidence, that is, results from different patient cohorts, or similar immunological status situation
• h: the comparator group is a historical control
• u: uncontrolled trial
• a: published abstract (presented at an international symposium or meeting)

Table 2. Risk factors for CMV resistance in HSCT recipients[2]

Risk Factors
Host factors
Prolonged antiviral CMV drug exposure (>3 months)
Previous antiviral CMV drug exposure
Recurrent CMV infection
Inadequate antiviral CMV drug absorption and bioavailability
Inadequate antiviral CMV oral prodrug conversion
Variation in antiviral CMV drug clearance
Subtherapeutic antiviral CMV drug level
Poor patient compliance with antiviral drug regimen
T-cell depletion
Haploidentical, allogeneic, or cord blood HCT
Delayed immune reconstitution
CMV-seropositive recipient and CMV-seronegative donor
Treatment with antithymocyte antibodies
Active GVHD
Young age
Congenital immunodeficiency syndromes
Viral factors
CMV viral load increase while receiving treatment (after >2 weeks of adequate dosing)
Failure of CMV viral load to fall despite appropriate treatment
Rise in CMV viral load after initial decline while receiving appropriate treatment
Intermittent low-level CMV viremia
High CMV viral loads

Table 3: Cytomegalovirus genes involved in resistance mutations[3,4]

Gene CMV	Function	Antiviral resistance
UL97	Protein Kinase DNA polymerase	GCV, VGCV
UL54	DNA polymerase	GCV, VGCV, FOS, CDV, BCDV
UL97/UL27	Competitive inhibitor of the union of ATP to the protein kinase UL97	MBV
UL56/UL51/UL89	Terminase complex Mature virions production	LMV

GCV: ganciclovir, VGCV: valganciclovir; FOS: foscarnet, CDV: cidofovir; BCDV: brincidofovir; MBV: maribavir; LMV: letermovir.

Table 4. Biological and clinical activity of anti-CMV alternative agents

Drug	Toxicity	Target	Mechanism of action	Resistances	In vitro activity	Metabolism
Maribavir	Alteration of taste and headache[5]	UL97	Inhibition of viral encapsidation and nuclear egress of viral particles[6,7]	Mutations in UL97 and UL27 [5,8]	CMV and VEB [7]	CYP3A4 [9]
Letermovir	Irrelevant	Viral terminase complex pUL56	Inhibition of viral DNA cleavage and packaging [10,11]	Mutations in pUL56 [12]	Specific to CMV [13]	Glucuronidation mediated by UGT1A1/1A3, CYP3A and CYP2D6 [(174)]
Brincidofovir	Diarrhea	Competes with deoxycytosine-5-triphosphate (dCTP) for viral DNA polymerase	Prevents further DNA polymerization and interrupts DNA replication	Mutations in UL54[14]	Herpesvirus, polyomaviru, adenovirus, papillomavirus, and varicella-zoster virus [15,16, 17, 18]	Renal
Leflunomide	Myelotoxicity, gastrointestinal and hepatic	Inhibition of protein tyrosine kinase activity [19] and inhibition of dihydroorotate dehydrogenase [20]	Interferes with virion assembly	Unknown	Unknown	CYP enzymes are involved in the metabolism of leflunomide only to a small extent. High enterohepatic recirculation
Artesunate	Prolongation of QT interval. Multiple drug interactions	No viral target	Inhibits cellular activation pathways of human cells used by CMV [21, 22]	None	Plasmodium and CMV [21, 22]	Induces CYP3A4 and CYP2C19 and inhibits CYP2D6 and CYP1A2
Sirolimus	Immunosuppression , proteinuria, hypophosphatemia, edema, delayed wound healing, dyslipidemia	No viral target	Indirect inhibition of host cell mTORC2 used by CMV for viral replication [23, 24]	None	CMV	Substrate of CYP3A4 and P-glycoprotein

SURVEY REPORT: Challenges in CMV Prophylaxis for allo-HSCT Recipients in Spain: Key Findings from the GETH-TC 2022 Survey.

Rationale for the Survey

Despite continuous progress in the field of allogeneic hematopoietic stem cell transplantation (allo-HSCT), cytomegalovirus (CMV) infection continues to significantly impact the morbidity and mortality associated with this procedure, placing a substantial burden on healthcare resources. Traditional management strategies, particularly periodic monitoring of CMV viremia and preemptive treatment (PET), may need changing following the recent approval of Letermovir (LMV), the first drug authorized for primary prophylaxis in CMV seropositive recipients.

LMV approval was granted in Spain in August 2021, though this was restricted to high-risk patient groups. In response to this shift, the Spanish Group of Hematopoietic Transplantation and Cellular Therapy (GETH-TC) conducted a survey in January 2022 to assess the speed of LMV prophylaxis implementation in the country and explore variations in critical aspects of its management between different centers. In this summary, we highlight the most significant findings.

Participating centers

A total of 17 adult allo-HSCT units participated in the survey (Figure 1). The median annual number of allo-HSCT procedures per center over the last 5 years was 47 (17–120).

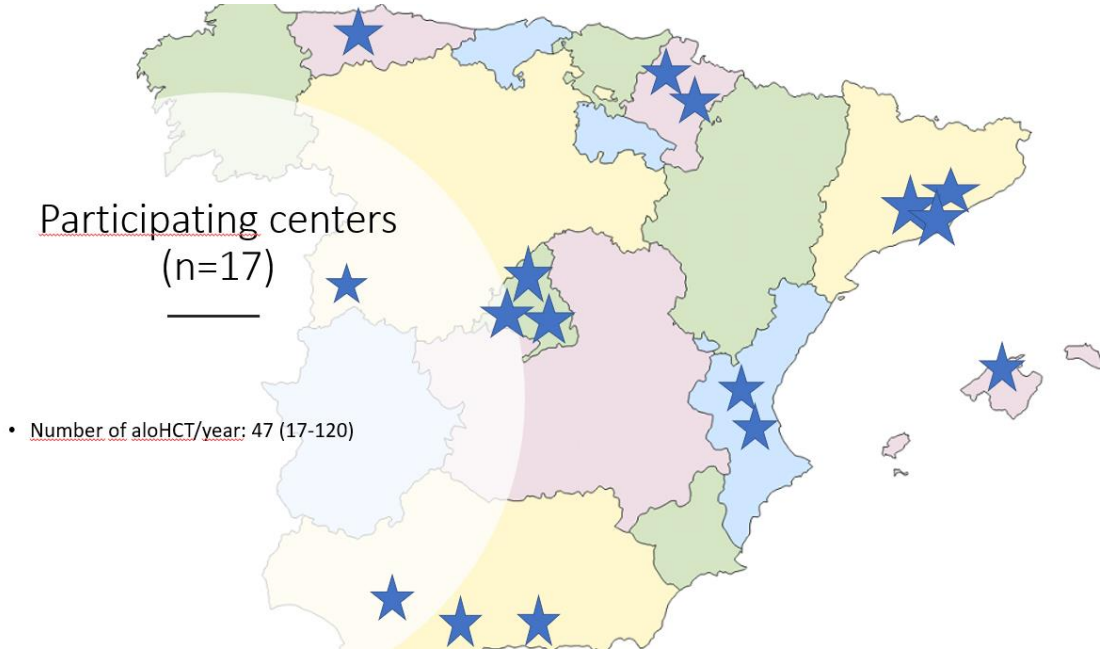

Figure 1. Geographical distribution of the participating centers. *Median number of allo-HSCT/year

Implementation of Letermovir prophylaxis in real clinical practice

As of January 2022, 14 of the 17 participating centers had already initiated the use of LMV (Figure 2). The remaining three centers were scheduled to begin its implementation within the next three months.

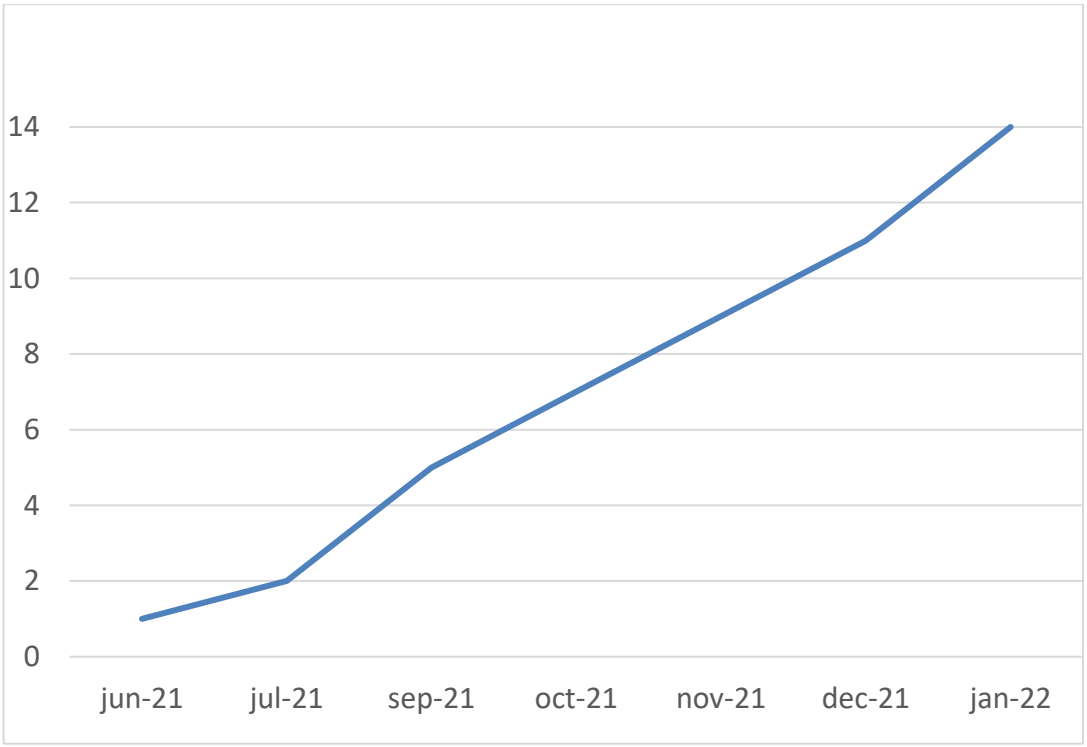

Figure 2. Implementation of LMV prophylaxis across the participating centers

At the time of the survey, 46% of allo-HSCT patients in the participating centers met the LMV funding criteria established by the AEMPS (Spanish Agency of Medicines and Medical Devices), with a range from 5 to 85% across different centers.

LMV was used as primary prophylaxis in 10 patients not initially considered at high risk, later identified as such based on other risk factors at the investigator's discretion and after compassionate use requests. Similarly, six cases involved the use of LMV as extended or secondary prophylaxis, with patient selection guided by clinical criteria without relying on specific immune response studies for decision-making.

Standardization of LMV Use in GETH-TC Centers

Only 59% of the participating centers (n=10) had a specific protocol for viremia monitoring and initiation of preemptive treatment (PET) when using LMV. In addition, significant variability in the definition of breakthrough infection was observed among these centers, with markedly divergent thresholds (Figure 3). It is notable that only two centers employed different threshold values based on the patient's baseline risk and incorporated the viremia doubling time into the decision-making process.

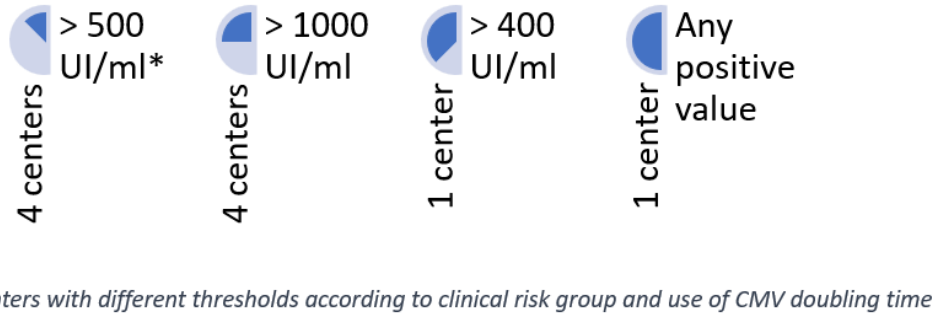

Figure 3. CMV DNAemia thresholds for initiation of PET across the participating centers

Availability of additional CMV-related techniques
In total, 82% of participating centers indicated having access to CMV antiviral resistance testing. However, in four of them (30%) samples had to be sent to external centers, leading to delays in obtaining results. Only 35% of studies routinely incorporated specific immunity against CMV screening and note that in two of these centers the test was unavailable on-site.

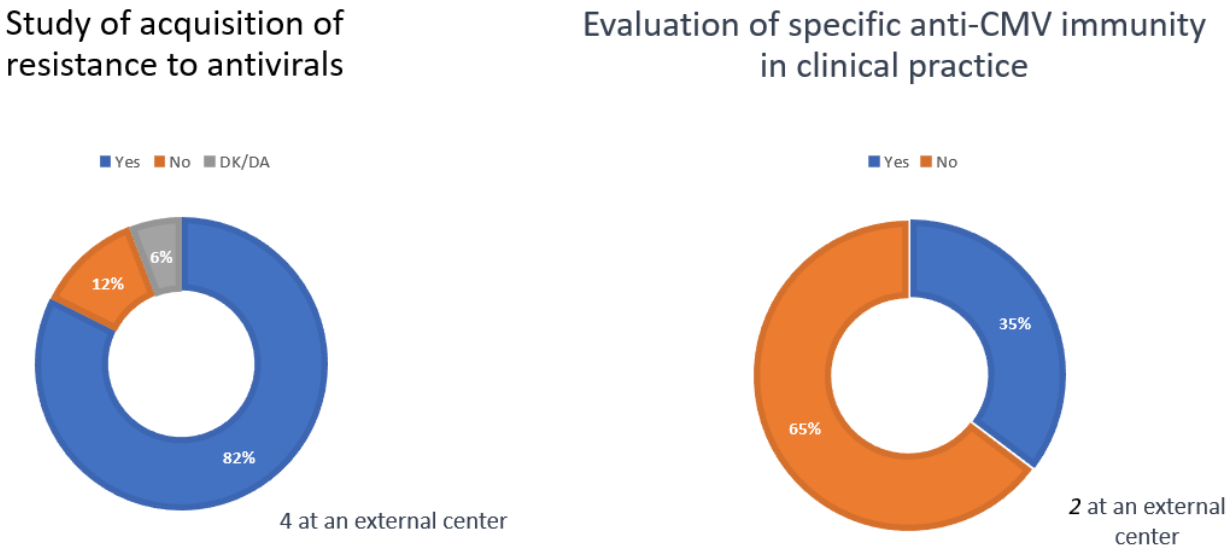

Figure 4. Availability of additional CMV-related techniques in participant centers

Viewpoint on the need for a national protocol to standardize LMV management.
There was unanimous consensus among participating centers of the need to create consensus guidelines and recommendations as a key aspect in the immediate future.

Conclusions
Following its approval, the implementation of LMV in GETH-TC centers has been rapid, although with varying rates and noticeable variability in the percentage of treated patients and usage beyond official recommendations. This situation introduces new challenges, requiring adjustments to traditional monitoring strategies and new criteria for precise differentiation between "blip" and breakthrough infection. It was also observed that a significant proportion of GETH-TC centers face obstacles in accessing important complementary tests, such as viral resistance studies, in a timely manner. This clinical guide has been updated in response to the unanimous consensus among all participating centers of the need to adopt standardized guidelines, with the primary goal of improving allo-HSCT outcomes in our environment.

References

1. Ullmann AJ, Cornely OA, Donnelly JP, et al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: Developing European guidelines in clinical microbiology and infectious diseases. *Clin Microbiol Infect*. 2012;18(SUPPL.7):1-8. doi:10.1111/1469-0691.12037

2. Chemaly RF, Chou S, Einsele H, et al. Definitions of Resistant and Refractory Cytomegalovirus Infection and Disease in Transplant Recipients for Use in Clinical Trials. *Clin Infect Dis*. 2019;68(8):1420-1426. doi:10.1093/cid/ciy696
3. Chou S. Advances in the genotypic diagnosis of cytomegalovirus antiviral drug resistance. *Antiviral Res*. 2020;176:104711. doi:10.1016/j.antiviral.2020.104711
4. Lopez-Aladid R, Guiu A, Sanclemente G, et al. Detection of cytomegalovirus drug resistance mutations in solid organ transplant recipients with suspected resistance. *J Clin Virol*. 2017;90:57-63. doi:10.1016/j.jcv.2017.03.014
5. Papanicolaou GA, Silveira FP, Langston AA, et al. Maribavir for Refractory or Resistant Cytomegalovirus Infections in Hematopoietic-cell or Solid-organ Transplant Recipients: A Randomized, Dose-ranging, Double-blind, Phase 2 Study. *Clin Infect Dis*. 2019;68(8):1255-1264. doi:10.1093/cid/ciy706
6. Biron KK, Harvey RJ, Chamberlain SC, et al. Potent and selective inhibition of human cytomegalovirus replication by 1263W94, a benzimidazole L-riboside with a unique mode of action. *Antimicrob Agents Chemother*. 2002;46(8):2365-2372. doi:10.1128/aac.46.8.2365-2372.2002
7. Williams SL, Hartline CB, Kushner NL, et al. In vitro activities of benzimidazole D- and L-ribonucleosides against herpesviruses. *Antimicrob Agents Chemother*. 2003;47(7):2186-2192. doi:10.1128/aac.47.7.2186-2192.2003
8. Chou S, Marousek GI. Accelerated evolution of maribavir resistance in a cytomegalovirus exonuclease domain II mutant. *J Virol*. 2008;82(1):246-253. doi:10.1128/JVI.01787-07
9. Goldwater DR, Dougherty C, Schumacher M, Villano SA. Effect of ketoconazole on the pharmacokinetics of maribavir in healthy adults. *Antimicrob Agents Chemother*. 2008;52(5):1794-1798. doi:10.1128/AAC.00951-07
10. Lischka P, Hewlett G, Wunberg T, et al. In vitro and in vivo activities of the novel anticytomegalovirus compound AIC246. *Antimicrob Agents Chemother*. 2010;54(3):1290-1297. doi:10.1128/AAC.01596-09
11. Melendez DP, Razonable RR. Letermovir and inhibitors of the terminase complex: a promising new class of investigational antiviral drugs against human cytomegalovirus. *Infect Drug Resist*. 2015;8:269-277. doi:10.2147/IDR.S79131
12. Goldner T, Hewlett G, Ettischer N, Ruebsamen-Schaeff H, Zimmermann H, Lischka P. The novel anticytomegalovirus compound AIC246 (Letermovir) inhibits human cytomegalovirus replication through a specific antiviral mechanism that involves the viral terminase. *J Virol*. 2011;85(20):10884-10893. doi:10.1128/JVI.05265-11
13. Marschall M, Stamminger T, Urban A, et al. In vitro evaluation of the activities of the novel anticytomegalovirus compound AIC246 (letermovir) against herpesviruses and other human pathogenic viruses. *Antimicrob Agents Chemother*. 2012;56(2):1135-1137. doi:10.1128/AAC.05908-11
14. Chou S, Ercolani RJ, Lanier ER. Novel cytomegalovirus UL54 DNA Polymerase gene mutations selected in vitro that confer brincidofovir resistance. *Antimicrob Agents Chemother*. 2016;60(6):3845-3848. doi:10.1128/AAC.00214-16
15. Williams-Aziz SL, Hartline CB, Harden EA, et al. Comparative activities of lipid esters of cidofovir and cyclic cidofovir against replication of herpesviruses in vitro. *Antimicrob Agents Chemother*. 2005;49(9):3724-3733. doi:10.1128/AAC.49.9.3724-3733.2005
16. Chemaly RF, Hill JA, Voigt S, Peggs KS. In vitro comparison of currently available and investigational antiviral agents against pathogenic human double-stranded DNA viruses: A systematic literature review. *Antiviral Res*. 2019;163:50-58. doi:10.1016/j.antiviral.2019.01.008
17. Gagelmann N, Ljungman P, Styczynski J, Kroger N. Comparative Efficacy and Safety of Different Antiviral Agents for Cytomegalovirus Prophylaxis in Allogeneic Hematopoietic Cell Transplantation: A Systematic Review and Meta-Analysis. *Biol Blood Marrow Transplant*. 2018;24(10):2101-2109. doi:10.1016/j.bbmt.2018.05.017
18. Ramsay ID, Attwood C, Irish D, Griffiths PD, Kyriakou C, Lowe DM. Disseminated adenovirus infection after allogeneic stem cell transplant and the potential role of brincidofovir - Case series and 10 year experience of management in an adult transplant cohort. *J Clin Virol*. 2017;96:73-79. doi:10.1016/j.jcv.2017.09.013
19. Xu X, Williams JW, Bremer EG, Finnegan A, Chong AS. Inhibition of protein tyrosine phosphorylation in T cells by a novel immunosuppressive agent, leflunomide. *J Biol Chem*. 1995;270(21):12398-12403. doi:10.1074/jbc.270.21.12398
20. Williamson RA, Yea CM, Robson PA, et al. Dihydroorotate dehydrogenase is a high affinity binding protein for A77 1726 and mediator of a range of biological effects of the immunomodulatory compound. *J Biol Chem*. 1995;270(38):22467-22472. doi:10.1074/jbc.270.38.22467
21. Efferth T, Marschall M, Wang X, et al. Antiviral activity of artesunate towards wild-type, recombinant, and ganciclovir-resistant human cytomegaloviruses. *J Mol Med (Berl)*. 2002;80(4):233-242. doi:10.1007/s00109-001-0300-8
22. Kaptein SJF, Efferth T, Leis M, et al. The anti-malaria drug artesunate inhibits replication of cytomegalovirus in vitro and in vivo. *Antiviral Res*. 2006;69(2):60-69. doi:10.1016/j.antiviral.2005.10.003
23. Moorman NJ, Shenk T. Rapamycin-resistant mTORC1 kinase activity is required for herpesvirus replication. *J Virol*. 2010;84(10):5260-5269. doi:10.1128/JVI.02733-09
24. Sarbassov DD, Ali SM, Sengupta S, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell*. 2006;22(2):159-168. doi:10.1016/j.molcel.2006.03.029