Drugs (2018) 78:1085–1103
https://doi.org/10.1007/s40265-018-0943-1

REVIEW ARTICLE

New Developments in the Management of Cytomegalovirus Infection After Transplantation

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Published online: 30 June 2018

Abstract

Cytomegalovirus (CMV) continues to be one of the most important pathogens that universally affect solid organ and allogeneic hematopoietic stem cell transplant recipients. Lack of effective CMV-specific immunity is the common factor that predisposes to the risk of CMV reactivation and clinical disease after transplantation. Antiviral drugs are the cornerstone for prevention and treatment of CMV infection and disease. Over the years, the CMV DNA polymerase inhibitor, ganciclovir (and valganciclovir), have served as the backbone for management, while foscarnet and cidofovir are reserved for the management of CMV infection that is refractory or resistant to ganciclovir treatment. In this review, we highlight the role of the newly approved drug, letermovir, a viral terminase inhibitor, for CMV prevention after allogeneic hematopoietic stem cell transplantation. Advances in immunologic monitoring may allow for an individualized approach to management of CMV after transplantation. Specifically, the potential role of CMV-specific T-cell measurements in guiding the need for the treatment of asymptomatic CMV infection and the duration of treatment of CMV disease is discussed. The role of adoptive immunotherapy, using ex vivo-generated CMV-specific T cells, is highlighted. This article provides a review of novel drugs, tests, and strategies in optimizing our current approaches to prevention and treatment of CMV in transplant recipients.

Key Points

Cytomegalovirus is a common opportunistic infection that adversely affects the outcomes of solid organ and allogeneic hematopoietic stem cell transplant recipients.

The CMV DNA polymerase inhibitor ganciclovir (and valganciclovir) serves as the first-line drug for CMV prevention and therapy, while foscarnet and cidofovir are reserved for the management of refractory and resistant CMV infection.

The viral terminase inhibitor, letermovir, is a newly approved drug for CMV prophylaxis after allogeneic hematopoietic stem cell transplant recipients.

Measurement of CMV-specific T-cell immunity is an emerging clinical tool with the potential to guide antiviral prophylaxis, pre-emptive therapy, and treatment of CMV disease after transplantation.

Adoptive CMV-specific T-cell therapy is emerging as an option for the treatment of CMV disease, especially that due to drug-resistant or refractory infections.

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1 Introduction

Cytomegalovirus (CMV) is a common infection that causes significant morbidity and mortality after solid organ transplantation (SOT) and allogeneic hematopoietic stem cell transplantation (HSCT) [1–4]. The virus has a widespread distribution worldwide, and depending on geography and socioeconomic status, CMV seroprevalence rates vary from 30 to 100%. In the USA, slightly over 50% of people are seropositive for CMV [5, 6]. During primary infection, CMV engages the innate and, later, adaptive immunity, and leads to development of neutralizing antibodies, and generation of CMV-specific T cells that play critical roles in effectively controlling infection [7–9]. However, the natural outcome of primary CMV infection is the establishment of lifelong subclinical latent infection in humans. Latent CMV can be detected in several cell types including monocytes, macrophages, lymphocytes, endothelial cells, and bone marrow progenitor cells. Reactivation of latent CMV in these cells occurs periodically throughout life in response to inflammation, stress, and immunosuppression. Such periodic reactivation of latent CMV is kept in check by the healthy immune system, which responds by progressive expansion of CMV-specific CD8 + T cells and, to a lesser extent, CD4 + T cells (referred to as memory inflation) [4, 10]. This immune fitness in healthy individuals limits the ability of CMV to cause significant clinical disease in immunocompetent hosts.

In contrast, CMV infection in immunocompromised individuals, such as SOT and HSCT recipients, can have devastating clinical consequences [1, 11–14]. In transplant recipients, CMV infection occurs most often during the first 3 months after transplantation, either as primary infection (in CMV-seronegative transplant recipients of organs and cells from CMV-seropositive donors, hereafter referred to as D +/R −), or as reactivation of latent infection (in CMV-seropositive recipients [R +]). Clinically, CMV infection can manifest with a wide array of clinical manifestations after transplantation. CMV disease in SOT recipients is commonly manifested as fever, body malaise, leukopenia, thrombocytopenia, and elevated liver enzymes (termed as CMV syndrome in SOT recipients). In both SOT and HSCT recipients, tissue-invasive CMV disease may occur, most often involving the gastrointestinal (e.g., gastritis, enteritis, colitis) [15] and respiratory (e.g., pneumonitis) tracts [16, 17]. Allograft infection is observed in SOT recipients, such that CMV hepatitis may be seen in liver recipients, nephritis in kidney recipients, and pneumonitis in lung recipients. CMV pneumonia is especially a morbid clinical illness in lung and HSCT recipients, with high rates of mortality. In addition, CMV is associated with numerous indirect effects, including a higher rate of acute rejection and chronic graft dysfunction after SOT, graft versus host disease after HSCT, and other opportunistic infections (e.g., *Pneumocystis jiroveci* pneumonia and pulmonary aspergillosis) and overall mortality after SOT and HSCT.

In this review, we provide an update on the new developments in the field of CMV management after SOT and HSCT. In particular, we discuss the role of letermovir, the newly approved drug, in CMV prevention after allogeneic HSCT recipients. We also highlight advances in CMV prevention strategies, both in terms of antiviral prophylaxis and pre-emptive therapy. Finally, we review the potential utility of immunologic monitoring in individualizing and optimizing our current approaches to CMV prevention and treatment.

2 Risk Factors for Cytomegalovirus (CMV) After Transplantation

Lack of effective CMV-specific immunity is the unifying characteristic that predisposes SOT and HSCT recipients to develop CMV infection and disease. In the clinical setting, CMV immunity is universally measured in all transplant candidates (and their donors) using serology to detect immunoglobulin G against CMV [18, 19]. Depending on pre-transplant CMV immune status, transplant recipients are categorized into high risk, moderate risk, or low risk (discussed in detail below). Immune assays that detect CMV-specific T cell immunity have also emerged in the clinical arena. Other risk factors for CMV infection and disease in SOT and HSCT are listed in Table 1. Knowledge of these risk factors is important in guiding the implementation of CMV prevention and treatment strategies.

2.1 Risk Factors for CMV in Solid Organ Transplantation (SOT) Recipients

CMV-seronegative SOT recipients lack pre-existing CMV-specific immunity, and they are at high risk of developing primary infection and clinical disease if they receive an organ from a CMV-seropositive donor (referred to as a CMV D + R − SOT recipient); the risk of CMV infection is, however, minimal if they receive an organ from a CMV-seronegative donor (CMV D −/R −) [18–21]. On the other hand, CMV-seropositive SOT recipients have pre-existing CMV-specific immunity that can suppress CMV reactivation, hence, their risk of CMV disease is considered moderate, and likely influenced by the intensity of pharmacologic immunosuppression. T-cell immune dysfunction is particularly intense with the use of lymphocyte-depleting drugs such as anti-lymphocyte globulin

Table 1 Risk factors for cytomegalovirus disease in transplant recipients [20, 21, 24, 45, 144, 145]

Immunologic status
CMV serologic status
CMV D+/R − in SOT recipients
CMV R+ in allogeneic HSCT recipients
CMV-specific T-cell immunity
Quantitative deficiency in CMV-specific CD4+ and CD8+ T cells
Functional defects in CMV-specific T cells (e.g., lack of interferon-gamma production, or cytotoxicity)
Global T-cell immunity
Low absolute lymphocyte count
Lack of response to mitogen (nonspecific antigen)
Other defects
Toll-like receptors, mannose binding lectin, others
Cytokine and chemokine defects
Allogeneic stimulation
Allograft rejection (in SOT patients)
Graft-versus-host disease (in HSCT patients)
Certain types of solid organ transplantation
Lung, small intestine, pancreas, and composite tissue are at high risk for SOT
T-cell-depleted stem cells and umbilical cord blood transplants for HSCT
Virologic factors
High absolute CMV viral load (peak viral load)
Rapid rise in CMV viral kinetics
Co-infection with HHV6 and HHV7
Host co-morbidity
Renal insufficiency
High blood transfusion requirements
Pharmacologic immunosuppression
T-cell-depleting agents
Antilymphocyte globulin
Muromonab-CD3
Antithymocyte globulin
Alemtuzumab
High-dose corticosteroids
High-dose mycophenolate mofetil
Myeloablative conditioning regimen for HSCT
Other immunosuppressive drugs (e.g., dasatinib)

CMV cytomegalovirus, *SOT* solid organ transplant, *HSCT* hematopoietic stem cell transplant, *HHV* human herpes virus, *D*+ donor positive, *R* − recipient negative, *R*+ recipient positive

(ALG), anti-thymocyte globulin (ATG), and alemtuzumab (anti-CD52 antibody) [22–24]. The use of these agents is characterized by a marked quantitative decline in CD3+, CD4+, and CD8+ T cells shortly after transplantation [25]. Functional impairment in T-cell function, as commonly measured by interferon-gamma release in response to CMV antigenic stimulation, is also impaired [26]. High-dose corticosteroids have also been associated with a higher incidence and increased severity of CMV disease [27]. Interestingly, use of mTOR inhibitors such as sirolimus and everolimus has been associated with a lower risk of CMV infection [28, 29]. Other risk determinants that may impair immune fitness, such as age (and immune senescence), medical co-morbidities, and inherent innate and adaptive immune defects contribute to CMV predisposition [30]. For example, single nucleotide polymorphism that impairs Toll-like receptor 2 (TLR2), an innate pattern recognition receptor that senses CMV, has been associated with CMV disease after liver transplantation [31, 32]. Likewise, polymorphisms of mannose-binding lectin increase the risk for CMV infection after transplantation [33, 34].

The risk of CMV infection and disease after SOT varies with the type of organ transplantation: lung, small bowel, and composite tissue allograft transplant recipients carry a higher risk of CMV disease compared to kidney, heart, and liver recipients; this may be explained by the intensity of immunosuppression and the larger amount of lymphoid tissue transplanted [23, 30]. Infection with human herpesvirus 6 and human herpesvirus 7 may predispose to CMV infection and disease [35–39]. Allograft rejection also increases the risk of CMV infection after SOT; the pro-inflammatory cytokines such as tumor necrosis factor-α that are released during acute rejection may reactivate CMV from latency [40], while treatment of rejection with intensified immunosuppression enhances viral replication by impairing the development of CMV-specific cell-mediated immunity [41].

2.2 Risk Factors in Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) Recipients

In contrast to SOT populations, CMV R+ patients have the highest risk of CMV infection after allogeneic HSCT [18, 19, 42]. In one study, CMV reactivation after allogeneic HSCT occurred in up to 80% of CMV D+/R+ recipients compared with only 30% of CMV D+/R − recipients [43]. The use of a myeloablative conditioning regimen "ablates" the pre-existing CMV-specific T-cell immunity of CMV R+ HSCT recipients, thereby leaving them immune-deficient when their endogenous latent CMV reactivates after transplantation. The intensity of myeloablation correlates directly with the risk of CMV; a standard myeloablative regimen confers a higher risk of CMV infection when compared to non-myeloablative conditioning regimens [18, 44]. In particular, total body irradiation and fludarabine-containing regimens increase the risk. Likewise, ATG depletes T-cell populations and enhances the risk of CMV infection. Some of the newer tyrosine kinase inhibitor drugs, such as dasatinib, have also been associated with CMV reactivation [45].

Transplantation of ex vivo T-cell depleted grafts or from an umbilical cord source is associated with higher CMV risk due to lack of transferred pathogen-specific immunity. Graft versus host disease (GVHD) and the use of mismatched or unrelated donors confer a higher risk of CMV infection, potentially due to the use of more intense immunosuppression, such as high-dose steroids, in these conditions. Finally, absolute lymphopenia, specifically low CD4 + T cell count and undetectable CMV-specific T-cell immunity, confer a high risk for CMV infection after transplantation [46–49].

2.3 Antiviral Drugs for CMV Prevention and Treatment

Antiviral drugs serve as the backbone for CMV prevention and treatment in transplant recipients. The antiviral drugs that are approved for management of CMV are intravenous ganciclovir, valganciclovir, foscarnet, cidofovir, and letermovir. Oral ganciclovir and fomivirsen are no longer clinically available (Table 2). A brief description of the currently available drugs is provided below.

The first-line drug for the management of CMV infection in transplant recipients is intravenous ganciclovir or oral valganciclovir. Foscarnet and cidofovir are considered as second-line agents that are reserved for treatment of resistant and refractory CMV. Letermovir is the newly approved drug for CMV prophylaxis in allogeneic HSCT recipients (Table 3).

In addition to the use of antiviral drugs, reduction in pharmacologic immunosuppression is strongly recommended, if this is feasible. Such an immune-minimization strategy will allow for reconstitution or recovery of CMV-specific T-cell immunity, which is critical for a durable clearance of infection [50], but this should be done cautiously so that it does not result in graft rejection after SOT, or GVHD in allogeneic HSCT recipients.

2.4 Ganciclovir and Valganciclovir

Ganciclovir was the first antiviral compound approved for CMV management. Three decades after its first use in the clinical setting, it remains the first-line drug for prevention and treatment of CMV after transplantation. It is available in oral (as valganciclovir) and parenteral (intravenous and intraocular) formulations; valganciclovir has 60% bioavailability. A valyl ester prodrug, valganciclovir is rapidly absorbed and metabolized to ganciclovir by intestinal and hepatic esterase [51]. Peak plasma concentrations are achieved in 1–3 h. Ganciclovir is excreted by glomerular filtration and active tubular secretion; dosage adjustment is required with impaired renal function.

Ganciclovir exerts its anti-CMV activity by inhibiting the function of CMV DNA polymerase. As an analogue of 2-deoxyguanosine, ganciclovir serves as a competitive substrate for *UL54*-encoded CMV DNA polymerase and its incorporation during viral DNA elongation results in premature termination of CMV DNA synthesis. For this process to occur, ganciclovir must be tri-phosphorylated; its initial phosphorylation to GCV monophosphate is catalyzed by CMV protein kinase (encoded by CMV *UL97*). Subsequent phosphorylation by host thymidine kinase results in active ganciclovir-triphosphate—a competitive substrate of CMV DNA polymerase.

The clinical uses of ganciclovir and valganciclovir for CMV prevention and treatment are discussed in detail below (sections on antiviral prophylaxis, pre-emptive therapy, and

Table 2 Antiviral drugs for cytomegalovirus prophylaxis and treatment

	Prophylaxis	Treatment	Main side effect
Preferred drugs			
Ganciclovir	5 mg/kg IV once daily	5 mg/kg IV every 12 h	Myelosuppression, especially leucopenia and neutropenia
Valganciclovir	900 mg PO once daily	900 mg PO twice daily	Myelosuppression, especially leucopenia and neutropenia
Letermovir	480 mg PO once daily (allogeneic HSCT recipients only [240 mg PO once daily if with cyclosporine])	Not recommended	Nausea and vomiting
Alternative drugs			
Valacyclovir	2 g PO four times daily (kidney recipients only)	Not recommended	Neurotoxicity
Foscarnet	Not recommended	60 mg/kg IV every 8 h or 90 mg/kg every 12 h	Nephrotoxicity; electrolyte disturbance; myelosuppression
Cidofovir	Not recommended	5 mg/kg once weekly × 2 weeks then every 2 weeks thereafter	Nephrotoxicity; myelosuppression

Table 3 Strategies for CMV prevention after solid organ and hematopoietic stem cell transplantation

Transplant group	Risk group	Prevention	Comments
Allogeneic HSCT	R +	CMV PCR surveillance with pre-emptive therapy of asymptomatic CMV reactivation Viral load threshold is variable Antiviral prophylaxis is an option Letermovir preferred IV Ganciclovir Valganciclovir	Pre-emptive therapy is the preferred approach Ganciclovir and valganciclovir is associated with myelosuppression, especially neutropenia Prophylaxis is associated with post-prophylaxis delayed-onset CMV infection and disease
Kidney	D +/R −	Antiviral prophylaxis for 6 months Valganciclovir IV Ganciclovir Valacyclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis is preferred but associated with post-prophylaxis delayed-onset CMV disease Valacyclovir (high-dose) is associated with neurotoxicity Valganciclovir is preferred drug for pre-emptive therapy
	R +	Antiviral prophylaxis for 3 months Valganciclovir IV Ganciclovir Valacyclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis and pre-emptive therapy are equally effective strategies
Pancreas	D +/R −	Antiviral prophylaxis for 3–6 months Valganciclovir IV Ganciclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis is preferred but is associated with post-prophylaxis delayed-onset CMV disease Valganciclovir is preferred for pre-emptive therapy
	R +	Antiviral prophylaxis for 3 months Valganciclovir IV Ganciclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis and pre-emptive therapy are equally effective strategies
Liver	D +/R −	Antiviral prophylaxis for 3–6 months Valganciclovir[a] IV Ganciclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis is preferred but is associated with post-prophylaxis delayed-onset CMV disease Valganciclovir is preferred drug for pre-emptive therapy
	R +	Antiviral prophylaxis for 3 months Valganciclovir IV Ganciclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis and pre-emptive therapy are equally effective strategies
Heart	D +/R −	Antiviral prophylaxis for 3–6 months Valganciclovir IV Ganciclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis is preferred but is associated with post-prophylaxis delayed-onset CMV disease Valganciclovir is preferred drug for pre-emptive therapy
	R +	Antiviral prophylaxis for 3 months Valganciclovir IV Ganciclovir CMV PCR surveillance and pre-emptive therapy is an option	Prophylaxis and pre-emptive therapy are equally effective strategies
Lung	D +/R −	Antiviral prophylaxis for at least 12 months Valganciclovir IV Ganciclovir Preemptive therapy is discouraged and not recommended	Prophylaxis is preferred but is associated with post-prophylaxis delayed-onset CMV disease
	R +	Antiviral prophylaxis for 6–12 months Valganciclovir IV Ganciclovir Pre-emptive therapy is discouraged and not recommended	Prophylaxis is preferred but is associated with delayed-onset CMV disease

IV intravenous, *CMV* cytomegalovirus, *PCR* polymerase chain reaction, *D +/R −* donor-positive/recipient-negative, *R +* recipient-positive

[a]NOT approved for CMV prevention in liver recipients, due to higher rate of tissue-invasive CMV disease in liver recipients, compared to the comparator product, oral ganciclovir (product package insert) [146]

treatment) (Table 3). The most common adverse effect of ganciclovir is bone marrow suppression, primarily neutropenia. Rash, pruritus, diarrhea, nausea, vomiting, and increased serum creatinine and liver enzymes have been observed. Neurotoxicity may occur [51].

2.5 Foscarnet

Foscarnet was approved as an anti-CMV drug, based on its efficacy for treatment of CMV retinitis in patients with

acquired immunodeficiency syndrome (AIDS). Foscarnet is also used as an alternative drug for treatment of CMV infection after transplantation [51]. Its oral absorption is poor, and it is only available in parenteral form. Foscarnet is not metabolized, and its plasma half-life is 3.3–6.8 h [52]. Foscarnet exerts its anti-CMV effect by inhibiting the function of CMV DNA polymerase. As a pyrophosphate analogue, foscarnet inhibits CMV DNA polymerase (encoded by *UL54*) by noncompetitively binding to its pyrophosphate binding site. This process blocks the cleavage of pyrophosphate from the incoming terminal nucleoside-triphosphate that is being added to the elongating DNA chain, thereby terminating CMV DNA synthesis.

In transplantation, foscarnet is reserved for CMV diseases that are resistant or refractory to ganciclovir. Such "alternate" role of foscarnet is due to common occurrence of renal dysfunction. Foscarnet-associated nephrotoxicity affects 30–50% of patients after long-term use [53]. This is caused by the deposition of foscarnet crystals in glomerular capillary lumen. Myelosuppression, mucosal ulcerations, and electrolyte disturbances such as hypocalcemia, hypomagnesemia, and hypophosphatemia are also common [54]. Patients should be hydrated and monitored routinely for serum creatinine and electrolytes during foscarnet use [51].

2.6 Cidofovir

Cidofovir was approved for CMV treatment based on studies in AIDS patients with CMV retinitis. Cidofovir is used as alternative treatment for CMV in transplant recipients. It has broad-spectrum activity against many DNA viruses, including CMV and other herpesviruses [55]. It is currently available only as a parenteral (intravenous) formulation, although an investigational oral form (brincidofovir) is undergoing clinical trials. The plasma half-life of cidofovir is 2.4–3.2 h, but it has a very long intracellular half-life (> 24 h), which allows for less frequent administration (every 1–2 weeks). Cidofovir is eliminated by glomerular filtration and tubular secretion.

Cidofovir exerts its anti-CMV effect by inhibiting CMV DNA polymerase. As an anhydrous synthetic acyclic deoxycytidine monophosphate analogue, it serves as a competitive substrate for DNA synthesis. Cidofovir is phosphorylated by host kinases into cidofovir-triphosphate that competes with deoxycytosine-5-triphosphate as a substrate for CMV DNA polymerase. Incorporation of cidofovir to the elongating DNA chain causes premature termination of viral DNA synthesis.

Cidofovir is reserved for the treatment of CMV diseases that are resistant or refractory to ganciclovir. Its use is complicated by a high rate of nephrotoxicity. Cidofovir binds to organic anion transporter with high affinity in the convoluted proximal tubules causing proximal tubular cell necrosis. The incidence and severity of nephrotoxicity may be reduced by hydration and probenecid, which is an inhibitor of organic anion transport [56].

2.7 Letermovir

Letermovir is a 3,4-dihydro-quinazoline-4-yl-acetic acid derivative that recently received approval for use as CMV prophylaxis in allogeneic HSCT recipients. Letermovir is available in oral and intravenous formulations. Letermovir is absorbed rapidly after oral administration, and its bioavailability is increased from 35 to 85% in the presence of cyclosporine [57]; the increased bioavailability is due to cyclosporine inhibition of hepatic transporters, including ATP-binding cassette transporters and soluble transporters [57]. The median time to maximum serum concentration is 45 min to 2 h. The mean terminal elimination half-life is 12 h [58]. It is eliminated through hepatic uptake; 93% of letermovir is excreted in feces. No dosage adjustment is needed for patients with creatinine clearance > 10 ml/min.

Letermovir exerts its anti-CMV activity by inhibiting CMV DNA terminase complex (encoded by *UL51*, *UL56*, and *UL89*). This terminase complex is required for CMV DNA processing and packaging (at a stage beyond DNA synthesis). By inhibiting terminase, letermovir prevents the cleavage of long DNA concatamers into individual viral units, thereby impairing the production of infectious particles [58–60].

Letermovir is approved for CMV prophylaxis in CMV R + allogeneic HSCT recipients (Tables 2, 3). It is not approved for CMV prevention in SOT recipients, or for treatment of asymptomatic CMV infection or CMV disease. Letermovir is highly specific for CMV and it has no activity against other herpesviruses [58]. The most common adverse effects of letermovir are nausea, vomiting, diarrhea, abdominal pain, peripheral edema, cough, and fatigue. There was concern that cardiac arrhythmias were associated with letermovir; however, this association was confounded by the patient's cardiac history [61]. Letermovir is not associated with myelotoxicity or nephrotoxicity.

3 Strategies for CMV Prevention

CMV prevention strategies are aimed to decrease end-organ disease and related mortality. The two main strategies for CMV prevention after transplantation are (1) antiviral prophylaxis and (2) pre-emptive therapy. Among the antiviral drugs discussed above, ganciclovir and valganciclovir are the most commonly used drugs for CMV prevention (Table 2). Letermovir is approved as CMV prophylaxis in

allogeneic HSCT recipients. In some instances, a hybrid approach is utilized wherein antiviral prophylaxis is used initially during the highest risk period, and then switched to CMV surveillance and pre-emptive treatment [23]. There are emerging investigational data to suggest that the implementation of prevention strategies may be optimized further by the incorporation of cell-mediated immunity monitoring, as discussed below.

3.1 Antiviral Prophylaxis

Antiviral prophylaxis implies the administration of an antiviral drug to transplant recipients at risk of CMV disease. The antiviral drug is administered shortly after transplantation, and continued for at least 3 months (or longer depending on transplant type and immune status) [62, 63]. Advantages of antiviral prophylaxis, specifically with the use of ganciclovir and valganciclovir, include the potential to prevent infections caused by other herpes viruses (herpes simplex virus (HSV), varicella zoster virus (VZV), and human herpes virus 6); this advantage is not seen with letermovir prophylaxis since it is highly specific for CMV, and does not have activity against other viruses (hence, additional anti-herpes prophylaxis is needed when letermovir is used for CMV prophylaxis). Antiviral prophylaxis has been associated with a lower incidence of CMV-related "indirect" effects such as allograft rejection, bacterial infections, protozoal infections, and mortality [64, 65]. Disadvantages of antiviral prophylaxis include the high cost of the drug, adverse toxicities (mainly leukopenia and neutrope-nia from ganciclovir or valganciclovir), and the occurrence of "post-prophylaxis" delayed-onset CMV (also referred to as late-onset CMV disease). This refers to CMV infection or disease that has been "delayed" by antiviral prophylaxis and it occurs most often during the first 3–4 months after completion of antiviral prophylaxis, particularly among patients who have not reconstituted CMV-specific T-cell immunity [64, 66].

3.1.1 Antiviral Prophylaxis in SOT Recipients

Clinical studies have consistently demonstrated the efficacy of antiviral prophylaxis in reducing CMV infection and disease after SOT, as summarized by a recent meta-analysis [65]. Valganciclovir (900 mg once daily; renally adjusted) is the most common drug for CMV prophylaxis after SOT. Intravenous ganciclovir (5 mg/kg intravenously once daily; renally adjusted) may be used in SOT patients unable to take oral medications (Table 2) [65]. Foscarnet and cidofovir are not recommended for CMV prophylaxis after SOT due to the common occurrence of adverse renal toxicities. Valacyclovir (2 g orally four times daily) is approved for CMV prophylaxis in kidney recipients, although neurotoxicity associated with high valacyclovir doses has limited its use [67]. Letermovir is currently being investigated in a randomized control trial that will compare it with valganciclovir for CMV prophylaxis in D + R − kidney recipients (NCT03443869).

The duration of antiviral prophylaxis after SOT continues to evolve, and vary depending on transplant type and severity of immunosuppression. It can be as short as 3 months (for low- to moderate-risk groups, such as CMV R + kidney or liver recipients) to as long as a minimum of 12 months (for highest-risk groups, such as CMV D +/R − lung recipients). In the past, the standard duration of valganciclovir prophylaxis was 3 months for "all at-risk groups" [68]. However, this was complicated by a high incidence of "post-prophylaxis" delayed-onset CMV disease [69]. A randomized clinical trial of 316 high-risk CMV D +/R − kidney recipients demonstrated that extending valganciclovir prophylaxis from 3 to 6 months significantly reduced the incidence of "post-prophylaxis" delayed-onset CMV disease (36.8 vs. 16.1%) [63]. This clinical trial serves as the basis for the current clinical practice guideline that recommends 6 months of antiviral prophylaxis to prevent CMV disease in CMV D +/R − kidney recipients [23]. Other non-lung SOT programs, such as heart, liver, intestinal, pancreas, and composite tissue allograft transplant programs, have adapted a similar approach by prolonging the duration of antiviral prophylaxis to 6 months in the highest risk group (D +/R −), despite lack of organ transplant-specific data (Table 3). Because the risk of delayed-onset CMV disease is low in CMV R + non-lung SOT patients, the duration of antiviral prophylaxis remains at 3 months for CMV R + kidney, liver, heart, and pancreas transplant recipients [23].

The transplant group at highest risk of CMV disease among SOT populations is the lung transplant group, hence the practice of longer duration of antiviral prophylaxis [70]. In one study, freedom from CMV infection and disease was significantly higher among lung recipients who received 180, 270, or 365 days of prophylaxis (90, 95, and 90%, respectively) compared to patients who received 100–179 days (64%) or < 100 days (59%) [70]. In a prospective, randomized, placebo-controlled study of 136 CMV D +/R − and R + lung recipients who completed 3 months of valganciclovir prophylaxis, extending the duration of valganciclovir prophylaxis to 12 months significantly reduced the incidence of CMV infection (64 vs. 10%, respectively) and disease (32 vs. 4%, respectively) [71]. The significant difference was sustained during the long-term follow-up of participants from a single center [72]. Accordingly, clinical practice guidelines recommend 12 months of valganciclovir prophylaxis for the CMV D +/R −, and 6–12 months for the CMV R + lung recipients [23]. Adjunctive CMV-specific immunoglobulin or intravenous immunoglobulin is also given to high-risk lung recipients in some centers. One meta-analysis suggested the incidence of CMV disease

was lower when CMV-specific immunoglobulin or intravenous immunoglobulin are included as part of prophylaxis, although this is debated [73].

Data from recent clinical studies suggest that the duration of antiviral prophylaxis in SOT recipients may be individualized, and guided by measurements of CMV-specific T-cell immunity (Table 4). A cohort of 124 CMV D+/R − SOT recipients was tested for interferon-gamma release in response to ex vivo stimulation with CMV antigens (using the QuantiFERON-CMV assay) at the end of valganciclovir prophylaxis and at 1 and 2 months thereafter. A quarter (25%) of patients developed CMV-specific CD8 + T-cell immunity during the testing period and were at significantly lower risk of "post-prophylaxis" delayed-onset CMV disease (incidence, 6.4%). The majority (65%) did not develop CMV-specific immunity during valganciclovir prophylaxis and were at a significantly higher risk of "post-prophylaxis" delayed-onset CMV disease (incidence, 22%). Ten percent of patients had an indeterminate result due to an impaired response to non-specific mitogen; this implies an over-immunosuppressed status, and these patients were at the highest risk of CMV disease (incidence, 58%) likely due to "overall" global suppression of CD8 + T cell function [74]. In another study of 95 patients who had enumeration of CMV pp65 and IE-1-specific CD69 +/interferon-γ-producing CD8 + and CD4 + T cells by flow cytometry at 30, 90, 120, 200, and 365 days after SOT, any detectable response at days 120 or 200 was protective against delayed-onset CMV disease [75]. These studies are two examples of many clinical studies that collectively indicate that measurement of CMV-specific cell-mediated immunity around the anticipated end date of antiviral prophylaxis may be a useful guide to predict the risk of delayed-onset "post-prophylaxis" CMV disease [74]. The information gathered from these tests may optimize and individualize the approach to preventing delayed-onset "post-prophylaxis" CMV disease—whether this is achieved through further extending the duration of antiviral prophylaxis or through another strategy is not known. In our clinical experience, the majority of CMV D+/R − SOT recipients will remain CMV immune-deficient during the period of valganciclovir prophylaxis, likely due to the lack of immune priming since valganciclovir is highly effective in suppressing CMV reactivation [76].

3.1.2 Antiviral Prophylaxis in Allogeneic HSCT Recipients

Antiviral prophylaxis has not been a first-line option for CMV prevention in allogeneic HSCT recipients because myelosuppression, particularly leukopenia and neutropenia, is commonly observed when using intravenous ganciclovir and valganciclovir (Table 3). In clinical trials of HSCT recipients, intravenous ganciclovir was highly effective in reducing CMV disease [77]. If used, ganciclovir is started after neutrophil engraftment, since its myelosuppressive effect may prevent engraftment or prolong the duration of neutropenia, which increases the risk for invasive bacterial

Table 4 CMV immunologic monitoring platforms and indications after transplantation [74, 75, 147–150]

	Platforms and indications	Comments and examples
Assays	Flow cytometry-based multimer or intracellular cytokine staining	Various laboratory developed tests Parameter measurement: intracellular cytokines and cell surface markers May differentiate CD4 + and CD8 + T cells May detect intracellular cytokines, most often interferon-gamma May detect cell activation markers
	Enzyme-Linked Immuno Spot (ELISPOT)	T.spot-CMV T-track CMV Parameter measurement: IFN-γ production by CD4 + and CD8 + T cells
	Interferon-gamma release assays (IGRA)	Measure cytokines (often, interferon-gamma) in samples activated by CMV antigens QuantiFERON-CMV Parameter measurement: IFN-γ production by CD4 + and CD8 + T cells
Clinical indications	CMV risk stratification	Predict the risk of: Post-transplant CMV viremia Post-prophylaxis CMV disease CMV disease progression Relapse or recurrence
	Guide initiation of pre-emptive therapy	Lack of adequate CMV-specific immunity may indicate need for treatment of asymptomatic CMV replication Adequate CMV-specific immunity characterize spontaneous clearance of asymptomatic CMV replication
	Guide duration of antiviral treatment	Adequate CMV-specific immunity may indicate sufficient antiviral therapy (optimal duration of treatment), with low risk of CMV relapse

CMV cytomegalovirus, *ELISPOT* enzyme-linked immunospot, *IGRA* interferon-gamma release assay, *IFN-γ* interferon-γ

and fungal infections [78]. Because of this, the most common method for CMV prevention after allogeneic HSCT is CMV surveillance and pre-emptive ganciclovir therapy (discussed separately below) [77]. Recently, letermovir was approved for use as prophylaxis to prevent CMV infection in allogeneic HSCT recipients, without untoward myelosuppressive effects.

The duration of antiviral prophylaxis is 100 days after allogeneic HSCT recipients, but this is expectedly associated the "post-prophylaxis" delayed-onset CMV disease. A multicenter, double-blind, placebo-controlled, randomized trial extended the duration from 100 days to 6 months of valganciclovir prophylaxis, and compared this with continued CMV surveillance- pre-emptive therapy in 184 allogeneic HSCT recipients. There was no significant difference in the incidence of the composite primary end point of death, CMV disease, or other invasive infections (20 vs. 21%). The secondary outcomes of CMV disease, CMV DNAemia, death, other infections, resource utilization, ganciclovir resistance, quality of life, immune reconstitution, and safety were also similar between the two approaches. There was a significant number of valganciclovir-treated patients who required hematopoietic growth factors (25.3 vs. 12.4%, $p = 0.026$) [79].

Letermovir was recently approved for CMV prophylaxis after allogeneic HSCT (Table 3). After successful Phase 1 and 2 studies, [80, 81] a Phase 3 clinical trial randomized 565 CMV R + HSCT recipients to letermovir (480 mg orally once daily [240 mg once daily in patients on cyclosporine]) or placebo for up to 14 weeks [61]. At 24 weeks, the primary end point of clinically significant CMV infection was significantly lower in patients who received letermovir prophylaxis (37.5 vs. 60.6%; $p < 0.001$). The significant reduction was observed even among the highest risk group, such as recipients of cord blood transplants, lymphocyte-depleted grafts, and those who had severe GVHD or were receiving high-dose steroids. The all-cause mortality rate was similar between letermovir and placebo at 48 weeks. There was no increased risk of myelotoxicity, hence circumventing the dreaded adverse toxicity that limited the use of valganciclovir prophylaxis after HSCT [61]. Notably, letermovir is highly specific for CMV and it has no activity against other herpes viruses; hence, additional strategies are needed (such as acyclovir prophylaxis) for the prevention of herpes simplex virus and varicella zoster virus.

Antiviral prophylaxis (either with valganciclovir or letermovir) is associated with delayed-onset "post-prophylaxis" CMV disease after HSCT. This is anticipated to occur in HSCT recipients with persistently impaired CMV-specific T-cell function. In the letermovir trial, the incidence of clinically significant CMV infection was 37.5%; these infections are predominantly of delayed onset (i.e., delayed by letermovir prophylaxis). Late-onset CMV infection is clinically relevant since it remains to be associated with non-relapse mortality after HSCT [82]. The optimal strategy to reduce the risk of "post-prophylaxis" delayed-onset CMV infection is not known, but should incorporate efforts to promote CMV-specific T-cell reconstitution [83].

3.2 Pre-emptive Therapy

With this strategy, antiviral drug is "selectively" given to SOT and HSCT recipients with asymptomatic CMV infection in order to "pre-empt" its progression to symptomatic clinical disease. This CMV-selective strategy will limit the use of antiviral drugs only to those who need it the most—those with evidence of active CMV replication. This strategy therefore requires strict monitoring of transplant recipients for viral replication using highly sensitive laboratory assays such as nucleic acid amplification tests at regular intervals. Guidelines suggested once-weekly testing for at least 3 months after transplantation (or longer depending on the period at highest risk), although others perform monitoring at twice a week or once every 2 weeks [23, 84]. The success of the pre-emptive therapy strategy is highly dependent on the adherence to this rigid monitoring schedule.

Pre-emptive antiviral therapy is initiated upon detection of CMV replication above a viral load threshold. However, there is no viral load threshold that is widely acceptable in various clinical settings [85]. Viral load thresholds vary depending on the type of assay (including size of amplicon), type of sample (plasma or whole blood), type of patient (SOT vs. HSCT, high-risk vs. moderate-risk), degree of immunosuppression, among others [86, 87]. Even with a standardized calibrator using the WHO International Standard, which was created to ensure harmony in CMV nucleic acid test reporting, there remains clinically important variability in viral load reporting [87]. It is therefore emphasized that, for each nucleic acid test and for every transplant patient group, a viral threshold is defined to guide pre-emptive therapy [23, 85].

Valganciclovir (900 mg orally twice daily; renally adjusted) is the antiviral drug recommended for pre-emptive treatment of asymptomatic CMV infection in SOT and HSCT recipients [23]. Intravenous ganciclovir (5 mg/kg every 12 h; renally adjusted) is reserved for patients unable to take oral medication. The duration of pre-emptive treatment is for at least 2 weeks, but this should be individualized based on viral load monitoring; antiviral treatment is continued until complete resolution of CMV replication (i.e., negative CMV test in the blood) [23]. It is recommended that two consecutive negative weekly tests be obtained before stopping treatment [23], but this recommendation may change with the use of more sensitive assays [88]. Occasionally, there will be missed cases of CMV disease that are not preceded by CMV viremia (i.e., compartmentalized diseases,

most often gastrointestinal CMV disease in CMV R + SOT). In addition, rapidly replicating CMV may be missed by weekly (or less frequent) surveillance and rapidly progress to cause tissue-invasive disease ("escape" infections) in high-risk CMV D +/R − patients [89].

There are emerging data that indicate the potential role of CMV immunologic monitoring in guiding the implementation of pre-emptive therapy (Table 4). The presence of effective CMV-specific T-cell immunity in a transplant patient with low-level CMV reactivation was associated with spontaneous viral clearance without the need for antiviral therapy [90]. As more clinical data emerge to support this finding, we anticipate the complementary use of viral load and immunologic monitoring in guiding pre-emptive therapy in transplant patients with asymptomatic CMV reactivation.

The advantages of pre-emptive therapy include lower drug toxicities and decreased drug cost. The savings associated with limited antiviral use are, however, offset by the cost of CMV surveillance. Allowing the occurrence of low-level CMV replication may theoretically prime the immune system to develop CMV-specific cell-mediated immunity, thereby lowering the risk of delayed-onset CMV infection [91, 92]. Since pre-emptive therapy is CMV-selective, it does not offer universal protection against other herpes viruses. Thus, when pre-emptive therapy is selected as the CMV prevention strategy, additional measures should be implemented to prevent other herpes viruses (i.e., acyclovir prophylaxis for herpes simplex virus). Pre-emptive therapy has not been consistently associated with a lower incidence of indirect effects, including mortality after SOT [65, 92].

3.2.1 Preemptive Therapy in SOT Recipients

Preemptive therapy is efficacious in preventing CMV disease after SOT. Valganciclovir (900 mg twice daily; renally adjusted) is as effective as intravenous ganciclovir (5 mg/kg every 12 h; renally adjusted) for the pre-emptive treatment of asymptomatic CMV infection (Table 3) [93]. In a retrospective study of SOT recipients, the kinetics of viral decline were not significantly different during pre-emptive treatment with valganciclovir ($t1/2 = 2.16$ days) or intravenous ganciclovir ($t1/2 = 1.73$ days; $p = 0.63$) [93]. In clinical practice, valganciclovir is the form that is most commonly used due to ease of oral administration. Foscarnet and cidofovir are not used for pre-emptive treatment because of nephrotoxicity, and they are given intravenously. Letermovir was compared to valganciclovir for pre-emptive treatment in a Phase 2 clinical trial of 27 kidney recipients with asymptomatic CMV reactivation [81]. After 14 days of treatment, there was no significant difference in the incidence of viral clearance between letermovir and standard-of-care treatment [81]. The dose of letermovir used in this small pilot study is lower than the dose approved for prophylaxis. However, letermovir is not currently approved for pre-emptive therapy, and there are no Phase 3 clinical trials planned to assess and demonstrate its efficacy for pre-emptive therapy.

Current guidelines recommend pre-emptive therapy with valganciclovir or intravenous ganciclovir as an option for prevention of CMV disease in moderate-risk non-lung SOT recipients (i.e., CMV R + heart, kidney, liver, and pancreas recipients). While it is not preferred, pre-emptive therapy is an option for CMV D +/R − heart, kidney, liver, and pancreas recipients if the rigorous approach to CMV surveillance can be implemented adequately [23]. Pre-emptive therapy is not recommended for the prevention of CMV disease in D +/R − and R + lung recipients [23].

Assessment of CMV-specific T-cell immunity at the onset of asymptomatic CMV replication may assist clinicians in deciding whether to initiate pre-emptive antiviral therapy (Table 4). In a prospective study of 37 SOT recipients who developed asymptomatic low-level CMV viremia [viral load, 1140 copies/ml (interquartile range 655–1542)], the presence of CMV-specific CD8 + T-cell immunity, as measured by interferon-gamma release, was associated with viral clearance even without antiviral treatment. Spontaneous viral clearance, in the absence of antiviral therapy, was significantly higher in patients with compared to those without CMV-specific cell-mediated immunity (92.3 vs. 45.5%; $p = 0.004$) [90]. Thus, CMV-specific cell-mediated immune assessment at the onset of asymptomatic CMV viremia may predict progression versus spontaneous clearance, thereby potentially being useful in refining current viral load-guided pre-emptive strategies.

3.2.2 Pre-emptive Therapy in HSCT Recipients

Pre-emptive therapy is the most commonly used strategy for the prevention of CMV disease after allogeneic HSCT [94]. It is highly effective, and it is preferred over valganciclovir prophylaxis (Table 3) [77]. Data from recent trials indicate that CMV reactivation may occur in over 50% of allogeneic HSCT recipients [79]. In the letermovir prophylaxis trial, 103 (60.6%) of 170 patients randomized to placebo developed clinically significant CMV infection [61]. The high incidence of CMV replication observed in these trials suggests that a CMV monitoring protocol (i.e., weekly CMV nucleic acid test) should be implemented and followed strictly so that pre-emptive antiviral therapy can be initiated promptly [79].

The most commonly used drugs for pre-emptive treatment of CMV replication in HSCT recipients are intravenous ganciclovir (5 mg/kg every 12 h; adjusted renally) or oral valganciclovir (900 mg twice daily; adjusted renally) [95]. Foscarnet has also been used for pre-emptive therapy after HSCT. In contrast, letermovir is not approved and should not be used for pre-emptive therapy of CMV replication.

The duration of treatment is at least 2 weeks, but should be extended until the durable resolution of CMV replication. In a recent clinical trial, only 1.8% of HSCT patients who underwent CMV surveillance and pre-emptive treatment developed tissue-invasive CMV disease, which commonly affected the gastrointestinal tract [61]; this incidence is much lower than the historical CMV disease rates of over 8.9% [96]. Hence, the incidence of CMV disease is low among allogeneic HSCT recipients undergoing CMV surveillance and pre-emptive therapy [97].

As predicted, pre-emptive valganciclovir or ganciclovir treatment is associated with myelotoxicity that may require use of hematopoietic growth factors (25.3 vs. 12.4%, $p=0.026$) [79]. Because of the risk of ganciclovir-associated neutropenia [95], pre-emptive therapy with foscarnet has been investigated after HSCT [98]. In a trial of 213 patients that compared treatment with foscarnet ($n=110$) or ganciclovir ($n=103$), the Kaplan–Meier estimates of event-free survival within 180 days after HSCT were similar. However, during treatment, severe neutropenia (< 500 cells) was more common with ganciclovir (11%) than foscarnet (4%; $p=0.04$). While impairment of renal function was not significantly higher, the risk of nephrotoxicity with foscarnet remains a major concern in the clinical setting. Hence, foscarnet is considered as a second-line drug, and reserved for patients intolerant to ganciclovir.

4 Treatment of CMV Disease After Transplantation

The first-line drugs for the treatment of CMV disease after SOT and HSCT are intravenous ganciclovir (5 mg/kg every 12 h; renally adjusted) and valganciclovir (900 mg orally twice daily; renally adjusted) [84]. The alternative drugs are foscarnet and cidofovir, which are generally reserved for patients unable to tolerate valganciclovir or intravenous ganciclovir due to toxicities, and those refractory and resistant to ganciclovir. Intravenous immunoglobulin or CMV-hyperimmune globulin has also been used as adjunct therapy for severe CMV diseases, such as CMV pneumonitis in lung and allogeneic HSCT recipients. A pooled analysis of clinical studies revealed that the addition of immunoglobulin may reduce CMV disease severity, particularly for CMV pneumonitis, in HSCT and mortality in SOT recipients [99, 100], although other studies do not show this benefit [101]. In addition to antiviral drugs, reduction in immunosuppression is recommended, especially for severe CMV disease cases, to allow for reconstitution of global and pathogen-specific immunity that is essential for durable clearance of CMV infection [50].

Intravenous ganciclovir (but not valganciclovir) is the recommended first-line drug for the treatment of severe, life-threatening CMV disease or when gastrointestinal absorption is a concern. For the treatment of mild to moderate CMV disease, however, valganciclovir was found to be similarly effective to intravenous ganciclovir [102]. A study of 326 SOT recipients with mild to moderate CMV disease compared the efficacy of valganciclovir (900 mg twice daily) or intravenous ganciclovir (5 mg/kg intravenously every 12 h) for 21 days, followed by secondary prophylaxis with valganciclovir (900 mg daily) for additional 28 days. The rates of viremia eradication were similar for valganciclovir and intravenous ganciclovir at day 21 (45.1 and 48.4%, respectively) and day 49 (67.1 and 70.1%, respectively). Clinical success was similar for both groups at day 21 (77.4 and 80.3%, respectively) and day 49 (85.4 and 84.1%, respectively) [63]. A similarly-designed study will be difficult to perform to assess the efficacy of valganciclovir in allogeneic HSCT recipients, because CMV disease is not common in the current era of effective pre-emptive therapy after HSCT [77]. Nonetheless, based on robust SOT data and the widespread experience with pre-emptive valganciclovir therapy in HSCT, valganciclovir and intravenous ganciclovir are considered the first-line treatments for CMV disease in HSCT recipients.

The duration of antiviral treatment of CMV disease should be individualized and guided by resolution of clinical symptoms and weekly viral load monitoring [23]. In a prospective study of 376 episodes of CMV disease after SOT, the median duration of antiviral treatment was 18 days for intravenous ganciclovir and 21 days for valganciclovir [102]. In another study of 267 SOT patients, a pretreatment CMV viral load < 18,200 IU/ml was 1.5 times faster to CMV disease resolution [103]. Likewise, CMV suppression to < 137 IU/ml at days 14 and 21 was predictive of faster clinical response to therapy. Current clinical practice guidelines recommend that multiple (at least two consecutive) weekly negative viral load results should be obtained before stopping antiviral treatment [23]. In addition, emerging data indicate that CMV immunologic monitoring can further optimize the duration of antiviral treatment (Table 4). In a study of 27 SOT patients being treated for CMV infection (median viral load, 10,900 IU/ml), the presence of CMV-specific cell-mediated immunity was associated with a lower risk of CMV relapse after discontinuation of antiviral treatment. In this study, only one of 14 patients with positive CMV-specific T-cell response had low-level asymptomatic recurrence of viremia. In contrast, nine of 13 patients (69%) without CMV-specific T-cell responses had relapse despite receiving 8 weeks of additional secondary valganciclovir prophylaxis [104]. This study suggests that CMV-specific cell-mediated immune measurement may be used to assess the risk of relapse, and may optimize the duration of antiviral treatment. However, the approach to prevention of CMV relapse is not defined. The occurrence of CMV relapse

despite secondary valganciclovir prophylaxis suggests that antiviral therapy alone is insufficient for preventing relapse. Importantly, failure to develop CMV-specific immunity despite exposure to high levels of CMV replication indicates the need to further reduce immunosuppression, thereby allowing for immune reconstitution to prevent CMV relapse [104]. However, a randomized controlled clinical trial to address the optimal approach for preventing relapse will need to be performed.

4.1 Resistant and Refractory CMV Disease

Drug resistance should be suspected when antiviral treatment does not lead to a decline in viral load by ≥ 1 log, or there is failure to achieve significant improvement in clinical symptoms despite 2 weeks of full-dose ganciclovir or valganciclovir therapy. These patients should have their CMV strain tested for the presence of *UL97* and *UL54* mutation using a genotypic test [23, 105].

Resistance or refractoriness to intravenous ganciclovir or valganciclovir treatment is observed most commonly among CMV D+/R − SOT patients with CMV disease. In a study of 65 CMV D+/R − SOT recipients with "post-prophylaxis" delayed-onset CMV disease, nine (14%) had refractory CMV disease, including four (6%) with *UL97* and *UL54* mutations that confer ganciclovir resistance [106]. Another retrospective case-control study of SOT recipients found a 4.1% rate of drug resistance among D+R − recipients, and this was associated with a higher mortality rate compared to CMV infection due to susceptible strains [107]. The degree of resistance to ganciclovir conferred by mutation in CMV *UL97* depends on the specific codon that is involved. The most common *UL97* mutations that confer high-level resistance to ganciclovir are M460V/L, H520Q, A594V, L595S, and C603W. Mutations in the *UL54* gene, which encode for CMV DNA polymerase, are much less common, and are often observed in combination with an already pre-existing *UL97* mutation. Isolated *UL54* mutation (in the absence of *UL97* mutation) is rare [108]. *UL54* mutations generally confer dual or triple cross-resistance with cidofovir and/or foscarnet [109].

Ganciclovir is more often cross-resistant with cidofovir than foscarnet. Hence, foscarnet is the drug of choice for treating ganciclovir-resistant or refractory CMV disease [53]. Foscarnet was used to treat refractory CMV infection and disease in 39 transplant recipients (22 SOT, 17 HSCT), including 15 with ganciclovir resistance mutations. After a median of 32 days of foscarnet treatment, the rate of virologic failure was 33%. Renal dysfunction was observed in 51% of patients by the end of foscarnet treatment. Cidofovir has also been used as a second-line agent for treatment of resistant and refractory CMV infection. Because ganciclovir associated *UL54* mutation more often confers cross-resistance, cidofovir is a less preferred antiviral regimen. In a retrospective study of 82 HSCT patients who received cidofovir treatment, including 47 patients who had previously received ganciclovir, foscarnet, or both drugs, only 26 patients improved after a median 22 days of treatment [110]. Renal dysfunction was observed in 21% of patients at the end of cidofovir treatment. Other smaller case series, however, demonstrated the clinical efficacy of cidofovir for treatment of CMV [111, 112]. Intravenous immunoglobulin or CMV-specific immunoglobulin as an adjunct to antiviral therapy has been used in treating drug-resistant CMV infection and disease [113]. The suboptimal clinical outcomes of foscarnet and cidofovir treatment of resistant and refractory CMV diseases warrant novel drug therapies and strategies.

5 Investigational CMV Drugs

5.1 Letermovir

Letermovir is only approved for CMV prophylaxis after allogeneic HSCT, but not in SOT recipients. A Phase 3 randomized double-blind trial comparing 28 weeks of prophylaxis with letermovir (480 mg once daily (or 240 mg once daily in the presence of cyclosporine) plus acyclovir) versus valganciclovir (900 mg once daily) is currently recruiting 600 CMV D+/R − kidney recipients. The aim of this study is to demonstrate noninferiority of letermovir for CMV disease prevention (NCT03443869).

Letermovir is not approved for pre-emptive therapy of asymptomatic CMV infection. In a Phase 2 trial, 27 kidney recipients with CMV replication were assigned to 14-day pre-emptive therapy with letermovir (40 mg twice a day or 80 mg once a day) or standard of care (commonly valganciclovir). All groups had significant decline in viral load from baseline (40 mg twice daily: $p = 0.031$; 80 mg QD: $p = 0.018$; standard: $p = 0.001$) [81]. However, no advance-phase clinical trial is planned to assess the efficacy of letermovir for pre-emptive treatment of asymptomatic CMV infection in SOT and HSCT recipients.

Letermovir is not approved for treatment of CMV disease, including that due to drug-resistant virus. Letermovir is active in vitro against CMV resistant to ganciclovir, foscarnet, and cidofovir [114]. Letermovir treatment in a lung recipient with refractory multidrug-resistant CMV disease resulted in rapid clinical, virologic, and radiologic resolution when it was used in combination with reduction in immunosuppression [114]. However, there are no controlled clinical trials planned for treatment indications. Letermovir possesses a low genetic barrier to resistance, which is concerning when used for treatment of high-viral burden CMV

infection [60]. Letermovir resistance has been mapped to mutations of *UL56*, and less commonly (in vitro) to mutations in *UL51* and *UL89* [115–118]. In the letermovir prophylaxis trial, one HSCT patient developed a breakthrough infection due to *UL56* V236M mutant CMV strain [61]. This *UL56* V236M mutation has previously been identified during a Phase 2 trial, when a patient developed breakthrough CMV infection during letermovir prophylaxis [116]. Other mutations in *UL51*, *UL56,* and *UL89* have been selected under experimental letermovir pressure conditions in vitro, resulting phenotypically in synergistic letermovir resistance [119].

5.2 Maribavir

Maribavir is an orally bioavailable benzimidazole riboside currently undergoing clinical trials for CMV prevention and treatment [120]. Maribavir directly inhibits CMV by competing with ATP for binding to *UL97* kinase [121]. After successful Phase 1 and 2 trials, maribavir failed to show efficacy in initial Phase 3 clinical trials. In one double-blind, multicenter, placebo-controlled trial of 681 allogeneic HSCT recipients, there was no difference in CMV disease rates at 6 months between maribavir (100 mg twice daily for 12 weeks) or placebo (4 vs. 5%) [122]. Another randomized trial observed that maribavir (100 mg twice daily) was less effective than oral ganciclovir (1 g three times daily) for preventing CMV disease in CMV D+/R − liver recipients. CMV infection or disease was significantly lower with oral ganciclovir compared to maribavir, both at day 100 (20 vs. 60%, $p < 0.0001$) and at 6 months (53 vs. 72%, $p = 0.0053$) after liver transplantation [123].

Despite these disappointing initial results, a case series of maribavir treatment of refractory and resistant CMV infection and disease suggests its potential clinical utility [124]. Hence, it was re-evaluated, using higher doses, in a Phase 2 clinical trial (NCT01611974) that enrolled 120 HSCT patients with refractory and resistant CMV infection. The proportions of patients with undetectable viremia at 6 weeks of treatment with different doses of maribavir (400, 800, and 1,200 mg orally twice daily) were similar (70, 62.5, and 71%, respectively) (data available at clinicaltrials.gov [NCT01611974] and presented at IDWeek 2016) [125]. Maribavir is now undergoing a Phase 3 CMV disease prevention trial (NCT02927067) that is anticipated to enroll 550 allogeneic HSCT recipients, who will be randomized to maribavir or valganciclovir for treatment of asymptomatic CMV replication. A Phase 3 maribavir treatment trial (NCT02931539) is also recruiting 351 transplant patients to compare maribavir with standard of care for the treatment of resistant or refractory CMV infection. The most common adverse effect is dose-related metallic or bitter taste. The other side effects are headache, nausea, diarrhea, rash, and fatigue [126].

5.3 Brincidofovir

Brincidofovir is an oral lipid formulation of cidofovir that is converted intracellularly, with cleaving of the lipid chain, into the active cidofovir diphosphate. It has similar activity to cidofovir against CMV and other double-stranded DNA viruses [55]. Unlike cidofovir, brincidofovir is not a substrate for organic anion transporters and, therefore, has significantly reduced potential for renal toxicity [127]. In a Phase 2 trial, brincidofovir (100 mg orally twice weekly) significantly lowered the incidence of CMV infection in 230 allogeneic HSCT recipients (10 vs. 37%; $p = 0.002$) [127]. In a subsequent Phase 3 trial that evaluated brincidofovir prophylaxis versus placebo in 458 allogeneic HSCT recipients [128], brincidofovir suppressed CMV infection during 14 weeks of drug administration (NCT01769170). However, the rate of CMV infections soared during a 10-week post-prophylaxis follow-up period. The rate of graft versus host disease was also significantly higher with brincidofovir compared to placebo. Oral brincidofovir may have caused gastrointestinal toxicity that mimicked GVHD, which was treated with augmented immunosuppression, thereby increasing the risk of CMV during the post-prophylaxis period [128]. As predicted, there was no significant nephrotoxicity associated with brincidofovir [127].

There are no active clinical trials evaluating brincidofovir for CMV prevention, as the manufacturer is evaluating a better formulation. Nonetheless, a prospective registry database (NCT02167685) is ongoing to evaluate the potential impact of prior treatment with brincidofovir on long-term outcomes, including late CMV disease and survival.

5.4 Leflunomide

Approved for treatment of rheumatoid arthritis, leflunomide has been used, as an off-label drug, for the treatment of refractory and resistant CMV disease. In vitro data suggest that leflunomide has activity against several viruses, including CMV [129]. Leflunomide inhibits protein kinase activity and pyrimidine synthesis. It has been used, in anecdotal case reports, for the treatment of ganciclovir-resistant or refractory CMV disease with variable results [130, 131]. However, leflunomide is not been approved clinically as CMV treatment, and there are no large randomized clinical trials planned to assess its efficacy and safety.

6 Immunotherapy for CMV Disease

As already emphasized above, CMV-specific T cells play a critically essential role in the control of CMV infection [4]. Lack of CMV-specific T cells is the unifying factor that predisposes to CMV disease, including those that are refractory, resistant, and delayed-onset diseases [132]. Hence, adoptive transfer of ex vivo generated donor-derived CMV-specific CD4 + and CD8 + T cells have been suggested as potential treatment of CMV disease, especially when toxicities of current antiviral drug therapies are limiting [132–135].

6.1 Adoptive CMV-Specific T-Cell Immunotherapy in SOT Recipients

Adoptive T-cell therapies for treatment of CMV infection and disease in SOT recipients have been documented mostly in case reports. In one report, a CMV D +/R − lung recipient developed ganciclovir-resistant CMV pneumonitis that did not respond to several courses of antiviral therapy [136]. After autologous ex vivo CD8 + CMV-specific T-cell infusion, there was rapid and sustained clearance of CMV to undetectable levels, with reconstitution of long-term CMV-specific immunity [136]. No allograft rejection was observed [136]. In two other case reports, a lung and a kidney transplant recipient were successfully treated for ganciclovir-resistant CMV diseases with CMV-specific T-cell infusion [137, 138]. The kidney recipient developed CMV-associated thrombotic microangiopathy-related glomerulopathy, which was resolved after receiving salvage therapy with CMV-specific T cells obtained from a donor bank.

Cellular adoptive immunotherapy has limited availability in the current era (and it is not available in most SOT centers). In addition, there is generally a long duration of time to generate adequate CMV-specific T cells for infusion. Moreover, CMV-specific T-cell infusions have been reported to be associated with adverse events, such as graft failure, microangiopathy, and secondary malignancy [134].

6.2 Adoptive CMV-Specific T-Cell Immunotherapy in HSCT Recipients

CMV-specific T-cell immunotherapy has been used more often in HSCT populations. Some centers have investigated its use as a prophylactic measure to prevent CMV infections in allogeneic HSCT recipients. In one study, prophylactic adoptive transfer of ex vivo generated donor-derived CMV-specific cytotoxic T cells was given on or after day 28 to nine CMV D +/R + and D + R − allogeneic HSCT recipients. Six of the nine had detectable CMV-specific T cells within a week of infusion. While two CMV R + patients developed CMV viremia, none of them required antiviral treatment. No immediate unfavorable adverse event was observed [135]. Another study examined donor lymphocyte infusion, containing memory T cells, to allogeneic HSCT recipients. Among 31 patients with absent CMV-specific immune reactivity at baseline, donor lymphocyte infection resulted in the expansion of CMV-specific T cells within 100 days in 20 patients (64.5%) [139]. Hence, infusions of low-dose donor memory T-lymphocytes may constitute a simple measure to prevent infections in transplantation [139].

CMV-specific T-cell immunotherapy has also been used as treatment of CMV infection in allogeneic HSCT recipients [140]. In a prospective multicenter phase I/II clinical trial, HSCT patients with refractory CMV infection received ex vivo CMV-specific T cells [141]. In this study, eight HSCT recipients who received T-cell depleted stem cells were given adoptive T-cell therapy from their CMV-seropositive stem cell donors; CMV epitope-specific T cells were detectable in all patients. Complete and partial virologic response rates were 62.5 and 25%, respectively [141]. Interestingly, the use of a third-party donor did not provide expansion of CMV-specific T cells in HSCT recipients who received stem cells from CMV-seronegative donors [141]. Another study from a single center examined the CMV-specific T-cell expansion of 32 HSCT patients with refractory CMV infection who received ex vivo CD8 + CMV-specific T cell and less CD4 + CMV-specific T cells. Twenty seven (84.4%) of 32 patients had a resolution of CMV infection within 4 weeks after adoptive T-cell transfer, and did not experience further viral recurrence. This was correlated with in vivo expansion of CMV-specific T cells and improvements in cytokine production and proliferation ability of CMV-specific T cells. However, the remaining five patients who developed CMV recurrence 4 weeks after transfer were unable to restore the quantity or function of CMV-specific T cells [142]. Another study reported that adoptive CMV-specific T-cell therapy resulted in a favorable clinical response in a series of seven HSCT recipients with CMV infection refractory to antiviral treatment [143]. Finally, another use of third-party donor virus-specific T cells for others viruses (BK virus, human herpesvirus 6, Epstein-Barr virus, and adenovirus) appears to have treatment benefits [140]. Adoptive CMV-specific T-cell immunotherapy is undergoing a Phase 2 CMV disease prevention or pre-emptive therapy trial that is anticipated to enroll 50 allogeneic HSCT recipients, who will be randomized to adoptive transfer of CMV/EBV-specific T cells or to standard for treatment of asymptomatic CMV infection (NCT02227641).

7 Conclusion

CMV continues to be one of the most important pathogens that affect the short-term and long-term outcomes of SOT and HSCT. The search for its optimal prevention and treatment strategies continues to evolve. Advances in therapeutic and

diagnostic modalities contribute to our current management of CMV infection and disease after transplantation. In this review, we discussed the potential role of letermovir in the context of current prevention strategies after allogeneic HSCT. We briefly highlighted novel therapies, such as maribavir, that are in late-phase clinical trials. The emerging role of CMV-specific cell-mediated immunologic monitoring to guide prevention and treatment strategies was discussed. Likewise, the promise of CMV-specific T cells for adoptive immunotherapy was emphasized. The integration of these novel antiviral therapies, standardized molecular and immunologic tests, and immunotherapeutics collectively advance the management of CMV in SOT and HSCT recipients.

Compliance with Ethical Standards

Funding No funding was received for the preparation of this review.

Conflict of interest Dr. Meesing has nothing to disclose. Dr. Razonable received research grants (funds given to institution) from Roche, Genentech, and Qiagen, and served on the advisory board/data and safety monitoring board of Merck, Novartis, and Astellas.

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