



# Moving Past Ganciclovir and Foscarnet: Advances in CMV Therapy

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## Abstract

**Purpose of Review** CMV DNA polymerase inhibitors such as ganciclovir and foscarnet have dramatically reduced the burden of CMV infection in the HCT recipient. However, their use is often limited by toxicities and resistance. Agents with novel mechanisms and favorable toxicity profiles are critically needed. We review recent developments in CMV antivirals and immune-based approaches to mitigating CMV infection.

**Recent Findings** Letermovir, an inhibitor of the CMV terminase complex, was approved in 2017 for primary CMV prophylaxis in adult seropositive allogeneic HCT recipients. Maribavir, an inhibitor of the CMV UL97 kinase, is currently in two phase 3 treatment studies. Adoptive immunotherapy using third-party T cells has proven safe and effective in preliminary studies. Vaccine development continues, with several promising candidates currently under study.

**Summary** No longer limited to DNA polymerase inhibitors, the prevention and treatment of CMV infections in the HCT recipient is a rapidly evolving field which should translate into improvements in CMV-related outcomes.

**Keywords** Cytomegalovirus · Hematopoietic cell transplant · Antiviral · Letermovir · Maribavir · Filiciclovir

## Introduction

In 1989, ganciclovir (GCV) became the first anticytomegalovirus (CMV) agent approved by the US Food and Drug Administration (FDA) for the treatment and prevention of CMV infection and disease, followed by foscarnet (FOS), cidofovir (CDV), and valganciclovir (Table 1). All of these agents target the CMV DNA polymerase encoded by the UL54 gene (pUL54) to ultimately inhibit viral DNA synthesis. While these agents have dramatically reduced the burden of CMV infection in the hematopoietic cell transplant (HCT) recipient [1], their use is often limited by toxicities such as myelosuppression and renal injury, and the development of resistance [2]. Therefore, agents with novel mechanisms of action and improved toxicity profiles are clearly needed. In 2017, letermovir became the first antiCMV agent with a mechanism of action other than inhibition of DNA polymerase activity to be

approved by the FDA. This review will discuss recent developments in CMV antiviral agents and non-pharmacological interventions that may augment the ability to prevent and treat CMV infections in HCT recipients.

## Letermovir

### Mechanism of Action and Pharmacology

CMV genomic replication involves a rolling-circle mechanism that produces multiple genomic units linked in a head-to-tail manner (concatamers) [3]. The viral terminase complex cleaves concatameric viral DNA into full-length genomes and then packages a single genome into the viral nucleocapsid as part of new virion formation [3]. The core terminase complex is comprised of the proteins pUL51, pUL56, and pUL89; all three proteins are necessary for terminase function [4, 5]. Targeting the terminase complex represents an attractive therapeutic option since host cellular DNA replication does not require terminase functions and all three terminase proteins are individually essential for viral replication [6]. The first terminase inhibitors were the benzimidazole D-ribonucleosides such as BDCRB and TCRB [3]. Clinical development was halted after preclinical studies demonstrated

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**Table 1** Agents licensed or in development for the treatment or prevention of CMV infection and disease

Agent	Status	Target	Route of administration	Major toxicities <sup>1</sup>	Major drug interactions <sup>2</sup>	Resistance mutations		Activity against other herpes viruses		
						In vitro			In vivo	
						In vitro	In vivo		In vitro	In vivo
Ganciclovir	Approved <sup>3</sup> (1989)	DNA polymerase (UL54)	IV	Myelosuppression	None	UL97, UL54	UL97, UL54	HSV, VZV, HHV-6		
Valganciclovir	Approved <sup>3</sup> (2001)	UL54	Oral	Same as ganciclovir	None	UL97, UL54	UL97, UL54	HSV, VZV, HHV-6		
Foscarnet	Approved <sup>3</sup> (1991)	UL54	IV	Nephrotoxicity, electrolyte wasting, GI	None	UL54	UL54	HSV, VZV, HHV-6		
Cidofovir	Approved <sup>3</sup> (1996)	UL54	IV	Nephrotoxicity, neutropenia, GI, ocular	None	UL54	UL54	HSV, VZV, HHV-6		
Letermovir	Approved <sup>3</sup> (2017)	Terminase (UL56, UL51, UL89)	IV, oral	None	Cyclosporine, voriconazole, tacrolimus, sirolimus, statins, ergot alkaloids	UL56, UL51, UL89	UL56	no		
Maribavir	In phase 3	UL97 kinase	Oral	Taste disturbance, GI	Tacrolimus, sirolimus	UL97, UL27	UL97	no		
Filiciclovir	Phase 1 completed	UL54	Oral	ND <sup>4</sup>	ND <sup>4</sup>	UL97, UL54	ND <sup>4</sup>	HHV-6		

<sup>1</sup> For full listing of toxicities, please refer to the Summary of Product Characteristics (SPC) for each agent if available

<sup>2</sup> Excludes overlapping toxicities with agents commonly used after HCT

<sup>3</sup> Approved by the US FDA (year of approval) for prevention and/or treatment

<sup>4</sup> ND, not determined

unfavorable in vivo metabolism [7]. Other terminase inhibitors such as GW275175X [7] and tomegelevir (BAY 38-4766) [8] were not brought to clinical trials.

Letermovir is a 3,4-dihydroquinazoline derivative discovered to have activity against CMV by high-throughput screening of a compound library [9]. The 50% effective concentration (EC<sub>50</sub>) is in the 0.004-μM range, with a selectivity index > 15,000 [9]. The identification of letermovir resistance mutations L241P and R369S in UL56 along with the finding that letermovir impaired the formation of proper unit-length viral DNA genomes indicated that letermovir’s mechanism of action involved targeting the terminase complex [10]. Due to its mechanism of action, letermovir retains activity against CMV strains resistant to DNA polymerase inhibitors but, unlike DNA polymerase inhibitors, letermovir does not exhibit significant activity against HHV-6, HSV, or VZV [9, 11].

Letermovir can be administered intravenously or orally, is highly (~99%) protein bound, and is eliminated via biliary excretion [12]. Letermovir exerts mild-to-moderate inhibitory effects on cytochrome P450 (CYP) 3A and increases exposure to tacrolimus, sirolimus, and cyclosporine [13, 14]; these require monitoring and dose adjustment as needed when co-administered with letermovir. The dose of letermovir should be reduced by 50% (from 480 to 240 mg/day) when co-administered with cyclosporine [13]. Letermovir reduces voriconazole exposure but does not appear to affect posaconazole [15, 16]. Letermovir is contraindicated in persons receiving ergot alkaloids and in persons receiving certain statins along with cyclosporine [17]. Although letermovir is well tolerated in the setting of mild-to-moderate hepatic and renal impairment, it should be used with caution in severe hepatic impairment (ChildPugh Class C) and insufficient data exist to guide dose adjustments if the creatinine clearance is < 10 mL/min [18, 19].

### Clinical Experience

Letermovir was safe and well tolerated in a phase 1 clinical trial [20]. A phase 2 study compared letermovir at doses of 60, 120, and 240 mg daily to placebo for the prevention of CMV reactivation in seropositive allogeneic HCT recipients [21]. The incidence of virologic failure, defined as either detectable CMV infection leading to discontinuation of the study drug and administration of preemptive therapy or the development of CMV end-organ disease, was lower in the 240-mg group (6%) than in the 120-mg group (19%), the 60-mg group (21%), and the placebo group (36%). No safety concerns were identified.

Letermovir prophylaxis was then evaluated in a phase 3, placebo-controlled study in CMV seropositive allogeneic HCT recipients [22••]. A subset of patients were categorized as CMV high-risk, including HLA-A, B, or DR mismatch related donor, HLA-A, B, C, and DRB1 mismatch unrelated

donor, haploidentical donor, cord blood transplant, ex vivo T cell-depleted graft, or graft-versus-host disease (GVHD) of grade 2 or greater requiring  $\geq 1$  mg/kg/day prednisone (or equivalent). Letermovir prophylaxis at 480 mg/day (240 mg/day if co-administered with cyclosporine) was begun at a median of 9 days after HCT and continued through week 14 post-HCT, and during this time, weekly CMV PCR monitoring was performed. Preemptive therapy was initiated upon detection of viremia according to local practice, with protocol-suggested viral load thresholds of 150 copies/mL in high-risk patients and 300 copies/mL in non-high-risk patients. Patients with detectable viremia prior to randomization were excluded from the primary efficacy analysis. Letermovir prophylaxis met the primary endpoint of reduction in clinically significant CMV infection (requiring initiation of preemptive therapy or CMV disease) compared with placebo at 24 weeks (17.5% vs 41.8%). Since CMV disease was uncommon in both groups, this endpoint was largely defined by reduction in the need for initiation of preemptive therapy; as such, the benefit of letermovir prophylaxis will depend on the viral load threshold for initiating preemptive therapy. Importantly, letermovir prophylaxis was associated with a statistically significant reduction in all-cause mortality at 24 weeks, with this benefit being predominantly among high-risk patients. As CMV disease was rare and no single predominant cause of death was identified [23•], the reason(s) for the mortality benefit at week 24 remain unclear. The reduction in mortality appeared to correlate with the prevention of CMV viremia, raising the hypothesis that the beneficial impact of letermovir prophylaxis may be related to preventing indirect negative effects of CMV infection [24–26]. Adverse events including gastrointestinal effects (nausea, diarrhea), myelotoxicity, and nephrotoxicity were similar in the letermovir and placebo groups. Based on these results, letermovir was approved by the FDA for primary CMV prophylaxis in adult CMV seropositive allogeneic HCT recipients [17].

## Resistance

The finding of letermovir resistance mutations in UL56 was important in elucidating its mechanism of action [10]. Subsequent in vitro studies identified multiple additional UL56 resistance mutations, typically located between codons 231 to 369 [27, 28]. Mutations in UL89 and UL51 conferring reduced susceptibility to letermovir have also been observed in vitro [29, 30]. Letermovir-resistant mutants do not display a significant growth defect compared to wild-type CMV, even with mutations which confer complete (> 3000-fold) resistance, such as at codon C325 of UL56 [27]. Resistance appears to evolve more rapidly in vitro compared foscarnet, indicative of a relatively low barrier to resistance [27].

A single case of breakthrough infection with a letermovir-resistant strain containing the UL56 V236M mutation during

low-dose (60 mg daily) letermovir occurred in the phase 2 prophylaxis study [31]. An analysis of resistance during the phase 3 prophylaxis study was performed, focusing on identifying resistance-associated mutations in UL56 primarily and UL89 secondarily [32•]. UL56 genotyping was successful in 50 out of 79 patients (63%) who received letermovir prophylaxis and experienced CMV infection through week 24. Four UL56 resistance mutations were identified in 3 patients (6% of 50 patients analyzed). These 3 patients represented 16.7% of patients ( $N=18$ ) who experienced CMV infection while receiving letermovir prophylaxis and for whom UL56 genotyping was successfully performed. One of the 3 patients was viremic at the time letermovir prophylaxis was initiated (viral load < 151 copies/mL) and another patient missed 5 doses of letermovir. Of the four UL56 resistance mutations identified, two were previously documented resistance mutations (V236M and C235W), and two were novel mutations (E237G and R369T) at positions previously demonstrated to confer resistance in vitro [27, 28]. There were no UL89 substitutions documented that had previously had been identified as conferring resistance in vitro [30]; UL51 was not analyzed. Outside of these studies, cases of breakthrough infection and disease with letermovir-resistant virus have been reported in adult and pediatric HCT recipients receiving letermovir primary or secondary prophylaxis [33–35].

## Outstanding Questions

With a novel mechanism of action and proven ability to safely and effectively prevent CMV infection after HCT, letermovir represents a substantial addition to the CMV antiviral armamentarium that should demonstrably improve CMV-related outcomes in HCT recipients. However, important questions remain that will require additional study, including:

1. Determining the optimal duration of letermovir prophylaxis. In the phase 3 prophylaxis study, clinically significant CMV infection developed in ~10% of patients (~20% in those at high risk of CMV) between week 14, when letermovir was discontinued, and week 24 [22••]. This raises the question as to whether a longer duration of prophylaxis may be of benefit, as was found for valganciclovir after high-risk (CMV D+/R-) solid organ transplant (SOT) [36]. A phase 3 clinical trial will compare 100 vs 200 days of letermovir prophylaxis in CMV seropositive allogeneic HCT recipients, with the primary outcome measure being clinically significant CMV infection through week 28 post-HCT (NCT03930615).
2. Further defining the benefit of letermovir prophylaxis in high-risk HCT populations. High-risk patients comprised 31% of the total study population in the phase 3 prophylaxis study, with haploidentical transplant recipients comprising 14.3%, cord blood recipients 4%, and ex vivo T

cell-depleted recipients 2.5% [22••]. Additional study is needed, with some data already emerging [37, 38], to define the relative benefit of letermovir prophylaxis in specific high-risk HCT recipients who were relatively underrepresented in the study but for whom the benefit of letermovir prophylaxis appeared greatest.

3. Determining whether there is a role for letermovir in preemptive therapy or treatment of CMV disease. The use of letermovir monotherapy for these indications is not currently recommended due to the lack of supporting data. Notably, in the phase 3 prophylaxis study, 48 patients with detectable CMV viremia (viral load in all cases < 1000 copies/mL) prior to randomization received letermovir, thereby essentially receiving letermovir as preemptive therapy; of those, approximately 33% had clinically significant CMV infection by week 14 [22••, 39]. Additionally, concerns exist about the relatively low barrier to resistance in vitro, and emerging reports describe the development of resistance when used in the setting of active infection [40–43]. An ongoing clinical trial (NCT03728426) will evaluate the safety and efficacy of letermovir as salvage treatment of CMV infection or disease.

Given their distinct mechanisms of action, the combination of letermovir with DNA polymerase inhibitors represents an attractive possibility for the treatment of CMV due to the potential for additive or even synergistic antiviral activity. In one study, the combination of letermovir with the DNA polymerase inhibitors ganciclovir, foscarnet, and cidofovir demonstrated only an additive, not synergistic, effect in vitro [44]. However, another study found a small degree of synergy between letermovir and brincidofovir, an oral prodrug of cidofovir [45]. More work, both clinical and in vitro, is required to address the potential utility of letermovir-based combination therapy.

4. Determining the safety and efficacy of letermovir in pediatric HCT recipients. Studies of letermovir to date have been limited to adult patients. A phase 2b study of letermovir in pediatric HCT recipients is underway (NCT03940586) in order to provide much needed information pertaining to optimal dosing, safety, and efficacy in this population.

## Maribavir

### Mechanism of Action and Pharmacology

Maribavir is an orally available, benzimidazole L-riboside ATP competitive inhibitor of the CMV UL97 kinase (pUL97) [46]. This mechanism of action was revealed

through selection of a resistant virus containing a mutation in UL97 [46]. pUL97 is a broadly acting kinase that phosphorylates viral and host cellular proteins [47]. Unlike the CMV DNA polymerase pUL54 or components of the terminase complex, pUL97 is not absolutely essential for replication in tissue culture [6]. Instead, mutant viruses deleted of the entire UL97 gene, or in which pUL97 kinase activity has been abrogated, are viable but display severe growth defects [6, 48–51]. The critical function(s) of pUL97 that contributes to efficient CMV replication and is affected by maribavir to result in inhibition of viral replication remains poorly defined. Maribavir inhibits viral egress from the nucleus to the cytoplasm through inhibition of pUL97-dependent phosphorylation of the nuclear lamina component lamin A/C [52], although the relative contribution of this to maribavir's overall antiviral activity remains to be determined.

The antiviral activity of maribavir is greatly affected by cell culture conditions, with an EC<sub>50</sub> in human embryonic lung (HEL) fibroblast cells of ~0.14 μM compared with ~13 μM in human foreskin fibroblast (HFF) cells [53]. The reason for this difference is not entirely clear, but one possibility is that cell conditions modulate the activity of cellular kinases which can compensate for loss of pUL97 activity in the presence of maribavir [53]. Indeed, the addition of cellular kinase inhibitors reduces the maribavir EC<sub>50</sub> in HFFs to values comparable to HELs [53]. Since maribavir inhibits pUL97 activity and ganciclovir depends on pUL97-mediated phosphorylation for its activity, maribavir and ganciclovir are antagonistic [53]. Maribavir retains activity against most CMV strains resistant to DNA polymerase inhibitors [46, 54, 55] but, similar to letermovir, is not active against other herpesviruses [55].

Maribavir is available only as an oral preparation and is ~30–40% absorbed after oral administration [56]. Based on studies in animals, maribavir is eliminated mainly by biliary excretion [56, 57]. Maribavir clearance is not affected by renal impairment [58]. Maribavir is not a significant inhibitor of major CYP enzymes and does not affect voriconazole exposure [59]. However, maribavir increases tacrolimus exposure by ~50% [60], and therefore monitoring of tacrolimus and sirolimus when co-administered with maribavir is recommended [59, 60, 61•].

### Clinical Experience

Phase 1 clinical trials of maribavir evaluating doses up to 1200 mg twice daily showed maribavir to be safe and well tolerated, with the most common side effects being taste disturbance and headache [56, 62]. Maribavir was then evaluated in a multicenter, randomized, double-blind, placebo-controlled, dose-ranging phase 2 prophylaxis study in adult CMV seropositive allogeneic HCT recipients [63]. The doses of maribavir evaluated were 100 mg twice daily, 400 mg once daily, and 400 mg twice daily to start at engraftment (between

14 and 30 days after HCT) and continue for a maximum of 12 weeks after HCT. The primary endpoint was the incidence and time to onset of CMV infection or disease. All doses of maribavir demonstrated reduction in CMV infection as determined by detection of pp65 antigenemia or DNA PCR. CMV disease occurred in only 3 participants, all of whom were randomized to receive placebo. Adverse events were more common in the group receiving 400 mg twice daily compared with lower doses of maribavir and placebo; these consisted primarily of taste disturbance (31%) and gastrointestinal disturbances (28%) (nausea, vomiting, abdominal pain, dysphagia). All doses yielded similar trough concentrations but peak plasma concentrations were  $\sim 2.5\times$  higher following 400 mg doses compared with 100 mg doses, and the 24-h drug exposure was greatest in those taking 400 mg twice daily.

A subsequent phase 3 study evaluated maribavir at 100 mg twice daily compared with placebo for the prevention of CMV infection and disease in allogeneic HCT recipients [64]. The dose chosen was based on the lack of a dose-dependent effect on the incidence of CMV infection and an increase in adverse effects noted at the highest dose of 400 mg twice daily in the phase 2 prophylaxis study [63]. Maribavir was begun following engraftment (median of 24 days post-HCT) and administered for 12 weeks after HCT. Disappointingly, maribavir prophylaxis failed to show a reduction in CMV infection or disease compared with placebo at either day 100 or 6 months after HCT. Similarly, maribavir prophylaxis at 100 mg orally twice daily did not meet noninferiority compared with oral ganciclovir for the endpoints of prevention of CMV infection or disease in high-risk (CMV D+/R-) liver transplant recipients [65]. The reason(s) for the failure of maribavir in both studies are unclear [66, 67]. With the negative results of maribavir prophylaxis in both HCT and SOT recipients, maribavir was not further pursued for CMV prophylaxis.

Contemporaneous with prophylaxis studies, maribavir was being used under individual emergency investigational new drug applications as salvage therapy in situations of resistant or refractory infection. An initial experience described 6 patients (5 SOT recipients, 1 HCT recipient) treated with salvage maribavir at a starting dose of 400 mg twice daily [68]. Five patients had proven CMV end-organ disease and 4 patients had ganciclovir-resistant strains. Viral loads at initiation of maribavir ranged from 7200 to 1,811,171 copies/mL. Four patients cleared viremia after 6–41 days of maribavir; one of these patients was receiving FOS concomitant with maribavir.

A randomized, double-blind phase 2 study in HCT and SOT recipients with resistant or refractory CMV infections was subsequently performed [61]. Patients were randomized to receive 400, 800, or 1200 mg maribavir twice daily for up to 24 weeks. Forty patients were randomized to each treatment dose arm; of these approximately 40% were HCT recipients. 64.2% had asymptomatic infection, and end-organ disease was present in 13.3%. The median viral load at baseline was 3.7

$\log_{10}$  copies/mL. Eighty-six patients (72%) achieved an undetectable viral load. CMV-associated clinical manifestations improved in 24/34 (71%) patients and resolved in 6/34 (18%) by week 6 of treatment. Twenty-five patients (29%) who cleared viremia subsequently experienced CMV recurrence while receiving maribavir; 13 (52%) of these were associated with the emergence of maribavir-resistant virus (discussed below). The recurrence rate was lower among HCT recipients (26%) than among SOT recipients (40%) despite similar rates of virologic clearance (70% vs 64%, respectively). All efficacy endpoints were similar across all doses. Altered taste was the most common adverse effect (65%) but this resulted in treatment discontinuation in only one patient. Overall, this study demonstrated that maribavir may be a valid option in the setting of resistant or refractory infection, but the high rate of recurrence while on therapy and the associated emergence of maribavir resistance represent cause for concern. A phase 3 study of maribavir in HCT and SOT recipients with resistant or refractory CMV infections is underway (NCT02931539).

In parallel, a phase 2, open label study comparing maribavir to valganciclovir as preemptive therapy (absence of symptomatic infection or end-organ disease) following HCT or SOT was performed [69]. Patients were eligible if they had a CMV DNA viral load of 1000 to 100,000 copies/mL in blood or plasma. Patients were assigned to receive oral maribavir 400 mg, 800 mg, or 1200 mg twice daily or valganciclovir at a 900 mg twice daily for weeks 1 through 3 followed by 900 mg once daily for up to 12 weeks. The primary efficacy endpoint was the response to treatment, defined as undetectable CMV DNA in plasma within 3 weeks or 6 weeks after the start of treatment. Overall, 62% and 79% of patients had an undetectable viral load within 3 weeks and 6 weeks of maribavir treatment, respectively, compared with 56% and 67% for valganciclovir. No dose-dependent effect of maribavir on clearance of viremia was observed. The percentage of patients with recurrence of CMV infection at any time during the trial period was similar between maribavir and valganciclovir (22% vs 18%). Similar to other studies, altered taste was the most common adverse effect of maribavir ( $\sim 40\%$ ), followed by other gastrointestinal adverse effects (nausea, vomiting, diarrhea). Myelosuppression was more common in those receiving valganciclovir. A phase 3 trial of maribavir 400 mg twice daily versus valganciclovir for the treatment of first episodes of asymptomatic CMV infection in HCT recipients with a plasma viral load of  $\geq 1365$  International Units (IU)/mL and  $\leq 91,000$  IU/mL is now underway, with the primary outcome measure being clearance of viremia by 8 weeks of treatment (NCT02927067).

## Resistance

The UL97 mutations V353A, L397R, T409M, and H411L/N/Y emerge in vitro during maribavir selection and confer

moderate-to-high level (9-fold to >200-fold) resistance [46, 70, 71]. The first report of resistance during clinical use developed in a patient receiving maribavir as salvage therapy for CMV infection and was associated with UL97 T409M and H411Y mutations [72]. Resistance was not documented in either the phase 2 or 3 prophylaxis studies in HCT recipients [63, 64]. Genotypic analysis of maribavir-breakthrough infections from the phase 2 salvage study revealed de novo resistance mutations in 13 of 25 (52%) (T409M in 10, H411Y in 3) patients; development of resistance was equal across all maribavir doses [61•].

In general, resistance mutations in UL97 that arise during ganciclovir or maribavir selective pressure do not confer cross-resistance to the other agent [54, 73]. Surprisingly, however, one patient in the phase 2 salvage therapy study was retrospectively found to have a novel UL97 mutation F342Y after prolonged ganciclovir exposure but prior to beginning maribavir [74]. This mutation was found to confer GCV resistance and, unique to UL97 mutations selected during GCV exposure, cross-resistance to maribavir (4.5-fold). The patient went on to develop a UL97 H411Y mutation and eventually failed maribavir therapy.

Mutations in another CMV gene, UL27, arise under maribavir selection in vitro and also during propagation of UL97-defective strains [75–77], suggesting that mutations in this gene represent a fundamental mechanism of compensating for lack of pUL97 kinase activity. However, mutations in UL27 confer low-grade (~2–3-fold) resistance to maribavir [75–77] and have not been identified during clinical use.

## Filiciclovir (Cyclopropavir)

### Mechanism of Action

Filiciclovir (formerly “cyclopropavir”) is a second-generation methylenecyclopropane nucleoside analog of 2'-deoxyguanosine [78]. Filiciclovir, similar to GCV, is a nonobligate chain terminator of DNA synthesis that requires initial phosphorylation by pUL97, followed by additional phosphorylation steps performed by cellular kinases to its active triphosphate form [79–82]. Filiciclovir EC<sub>50</sub> values for CMV strains are approximately 0.2–0.3 μM, which are ~5-fold less than for GCV [78, 83–85]. The increased potency of filiciclovir compared with GCV in vitro may reflect the findings that filiciclovir is a better substrate for pUL97 than GCV [80, 86] and the CMV DNA polymerase incorporates filiciclovir-triphosphate into DNA more efficiently than GCV-triphosphate [82]. Filiciclovir displays little cytotoxicity at concentrations required to inhibit CMV replication in a variety of cell types [81] and demonstrated efficacy in a mouse model of CMV infection [87]. In addition to CMV, filiciclovir is active against HHV-6 but not HSV1, HSV2, or VZV [81].

In single-dose studies, oral bioavailability in rats and dogs ranged from 22 to 46% and 70 to 91%, respectively [88]. In single-dose rat toxicology studies, filiciclovir was well tolerated up to 300 mg/kg [88]. Preclinical studies and data from a single-dose (range 35–1350 mg) human study suggest that filiciclovir is primarily eliminated via renal excretion [89]. An L-valine ester prodrug, valcyclopropavir, with 95% bioavailability in mice was synthesized [90] but has not been further developed for clinical use at this time.

### Clinical Experience

A phase 1b ascending dose (100 mg, 350 mg, or 750 mg once daily for 7 days) trial was conducted in normal, healthy volunteers [89]. No serious adverse events were reported. Drug exposure plateaued around the 350 mg dose. The mean plasma concentrations exceeded the CMV in vitro 90% inhibitory concentration (IC<sub>90</sub>) for doses ≥ 100 mg per day.

### Resistance

Filiciclovir selection in vitro generates resistance mutations at canonical UL97 GCV resistance sites M460, H520, and C603 [2, 83, 85]. Filiciclovir also selects for novel UL97 mutations at positions F342 and V356, both of which individually confer cross-resistance to GCV and maribavir [51, 91]. Resistance mutations in the UL54 DNA polymerase also emerge under filiciclovir selective pressure in vitro, some of which result in cross-resistance to GCV and/or FOS [85].

Conversely, filiciclovir has been assayed against a variety of genotypically defined resistant CMV strains. Mutation at UL97 codon L595, one of the residues commonly involved in GCV resistance [2], confers no filiciclovir resistance [83, 91]. However, mutations at the other canonical GCV resistance sites including M460, H520, C592, A594, and C603 [2] result in 3–20-fold increases in filiciclovir EC<sub>50</sub> values [83, 91]. Thus, cross-resistance between filiciclovir and GCV and/or FOS may occur depending on the site of mutation.

### Brincidofovir

Brincidofovir (CMX001) is an oral lipid conjugate formulation of cidofovir with potent CMV activity [92]. In a phase 3 study in CMV seropositive allogeneic HCT recipients, brincidofovir prophylaxis for 14 weeks post-HCT did not meet the primary endpoint of prevention of CMV infection at week 24 compared with placebo [93•]. Brincidofovir was associated with significant gastrointestinal toxicity including acute GVHD and diarrhea [93, 94]. As such, oral brincidofovir is not being further developed as an antiCMV agent.

## Vaccine Development

In 1999, the Institute of Medicine, now the National Academy of Medicine, designated CMV as a highest priority for vaccine development [95]. This has proven a challenge, and there are no vaccines currently available for use. ASP0113 was a DNA vaccine encoding glycoprotein B (gB), which is capable of eliciting neutralizing antibodies, and the tegument protein pp65, which is a primary target of T cell responses [96, 97]. Unfortunately, ASP0113 failed to meet primary (overall mortality, CMV disease) or secondary (time to viremia and use of preemptive therapy) endpoints in a placebo-controlled, phase 3 study in HCT recipients [96]. ASP0113 also failed to meet the primary endpoint of reducing the risk of viremia through 1 year after transplant compared with placebo in a phase 2 study in CMV D+/R- renal transplant [98].

More recently, vaccine development has focused on incorporating the pentameric complex [96, 99]. The pentameric complex consists of gH/gL/pUL128/pUL130/pUL131, is required for CMV entry into several clinically relevant cell types, and elicits potent neutralizing antibody responses that block entry into those cells [96, 99]. A CMV vaccine candidate (V160) incorporating the pentameric complex was constructed from the live attenuated CMV AD169 strain that was further engineered to be replication-defective in the absence of a synthetic compound called Shield-1 [100]. Recently, this vaccine was found to be safe and elicited robust levels of neutralizing antibodies and T cell responses when administered to CMV-seronegative subjects in a phase 1 study [101, 102]. Several other candidate vaccines are currently being evaluated in phase 1 and 2 trials in adult and pediatric HCT recipients [96].

## Adoptive Immunotherapy and Passive Immunization

Adoptive immunotherapy denotes the reconstitution of CMV-specific T cell responses via the isolation, *in vitro* propagation, and transfusion of donor T cells to the recipient [103–106]. Adoptive immunotherapy has been safely used in HCT recipients as an adjunct to antiviral therapy for preemptive therapy and for the treatment of refractory CMV infection, and prophylactically after HCT, all in relatively small series [105, 107–114].

However, the need to generate specific T cell lines for each individual patient imposes logistical limitations for broad or immediate, time-sensitive use [115]. Using partially HLA-matched, banked third-party cells addresses these limitations [115]. The safety and tolerability of this approach in the management of refractory CMV infection or disease has been demonstrated in several nonrandomized studies [115, 116, 117]. The majority of patients in these studies exhibited

clinical and/or virologic responses following T cell infusion along with continued antiviral therapy. Thus, the incremental benefit of the transfused T cells is unclear. Randomized studies are now needed to definitively assess the benefit and safety of adoptive immunotherapy for the prevention or treatment of CMV infection in the HCT recipient [118].

The utility of intravenous immune globulin (IVIG) or CMV-enriched IgG in the management of CMV disease is unclear due to the lack of prospective, randomized trials evaluating the benefit of adjunctive IVIG compared with antiviral therapy alone. While not useful in the setting of gastrointestinal disease [119], the addition of IVIG to antiviral therapy in the management of pneumonia resulted in improved survival rates compared with historical controls in small studies [120–122]. However, a more recent, large retrospective analysis failed to demonstrate such a benefit [123] and therefore, the role of IVIG in the management of CMV pneumonia remains poorly defined. IVIG is not effective as prophylaxis in seronegative or seropositive HCT recipients [124–132].

A monoclonal antibody preparation that targets both the CMV glycoprotein B (gB) and the pentameric complex is in development [133]. A previous CMV monoclonal antibody that targeted the CMV gH protein (MSL-109) failed to demonstrate benefit when used as prophylaxis in HCT recipients [134].

## Conclusions and Future Directions

The CMV DNA polymerase inhibitors GCV, FOS, and CDV, while critical developments in reducing the morbidity and mortality associated with CMV infection in HCT recipients, are marked by issues of toxicity and resistance that often limit their use. The approval of letermovir—a nontoxic, orally available agent with a mechanism of action distinct from DNA polymerase inhibition—represents an important step in expanding the options for CMV prevention and towards the greater goal of improving outcomes after HCT. Additionally, the success of letermovir validates terminase inhibitors as a clinically relevant class of antiviral agents and may open the door to the development of other terminase inhibitors [135].

As agents with novel mechanisms of action such as letermovir and possibly maribavir are brought to clinical use, combination therapy for the treatment of CMV infection and disease becomes, for the first time, a possibility. *In vitro* studies generally support at least an additive effect, if not a synergistic one, of combining letermovir with DNA polymerase inhibitors or maribavir. Clinical studies are now needed to determine whether combination therapy for CMV is superior to monotherapy, as is true for the treatment of viral infections such as human immunodeficiency virus and hepatitis C virus [136, 137]. With no other agents besides maribavir and filociclovir currently in human studies, combination therapy

with existing agents and perhaps with indirectly acting antiCMV agents approved for other indications [138–153] that are unsuitable for use as monotherapy should be considered.

Advances in non-pharmacologic interventions will also be important in mitigating the impact of CMV infection. The safety of third-party T cells for use in adoptive immunotherapy demonstrated in initial studies moves this therapeutic intervention further towards becoming a realistic, viable option for more patients. The development of a safe and effective CMV vaccine remains a challenge but promising candidates are in development.

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## Compliance with Ethical Standards

**Conflict of Interest** The author declares no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not directly involve studies with human participants or animals performed by the author.

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