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Clinical development of letermovir and maribavir: Overview of human cytomegalovirus drug resistance

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ABSTRACT

The prevention and treatment of human cytomegalovirus (HCMV) infections is based on the use of antiviral agents that currently target the viral DNA polymerase and that may cause serious side effects. The search for novel inhibitors against HCMV infection led to the discovery of new molecular targets, the viral terminase complex and the viral pUL97 kinase. The most advanced compounds consist of letermovir (LMV) and maribavir (MBV). LMV inhibits the cleavage of viral DNA and its packaging into capsids by targeting the HCMV terminase complex. LMV is safe and well tolerated and exhibits pharmacokinetic properties that allow once daily dosing. LMV showed efficacy in a phase III prophylaxis study in hematopoietic stem cell transplant (HSCT) recipients seropositive for HCMV. LMV was recently approved under the trade name Prevymis™ for prophylaxis of HCMV infection in adult seropositive recipients of an allogeneic HSCT. Amino acid substitutions conferring resistance to LMV selected *in vitro* map primarily to the pUL56 and rarely to the pUL89 and pUL51 subunits of the HCMV terminase complex. MBV is an inhibitor of the viral pUL97 kinase activity and interferes with the morphogenesis and nuclear egress of nascent viral particles. MBV is safe and well tolerated and has an excellent oral bioavailability. MBV was effective for the treatment of HCMV infections (including those that are refractory or drug-resistant) in transplant recipients in two phase II studies and is further evaluated in two phase III trials. Mutations conferring resistance to MBV map to the *UL97* gene and can cause cross-resistance to ganciclovir. MBV-resistant mutations also emerged in the *UL27* gene *in vitro* and could compensate for the inhibition of pUL97 kinase activity by MBV. Thus, LMV and probably MBV will broaden the armamentarium of antiviral drugs available for the prevention and treatment of HCMV infections.

Overview of infections caused by human cytomegalovirus

Human cytomegalovirus (HCMV) is responsible for benign infections in immunocompetent adult individuals. On the other hand, HCMV is the leading cause of morbidity and mortality in immunocompromised patients such as transplant recipients and human immunodeficiency virus-infected individuals. Following a primary infection, HCMV establishes latency and persists for life into the host. HCMV primary infection can occur in seronegative transplant recipients from a seropositive donor. Viral reactivation or reinfection can occur in seropositive transplant recipients. HCMV disease can involve lungs, gastrointestinal tract, liver, retina and central nervous system or presents as a systemic syndrome. HCMV infection can also lead to indirect effects on the immune system including increased risks of infections caused by other pathogens, acute graft rejection and graft *versus* host disease. Strategies to prevent HCMV infection and disease include the administration of a prophylactic antiviral treatment to all at risk patients or the initiation of a preemptive antiviral treatment when the blood viral DNA load reaches a certain threshold. Until recently, all antiviral agents available for the prevention or treatment of HCMV disease targeted the viral DNA polymerase and were associated with potentially serious side effects.

1. Approved antiviral agents targeting the viral DNA polymerase

1.1. Mechanism of action of approved antiviral agents

Three antiviral agents and a prodrug are currently-available for the systemic treatment of human cytomegalovirus (HCMV) infections

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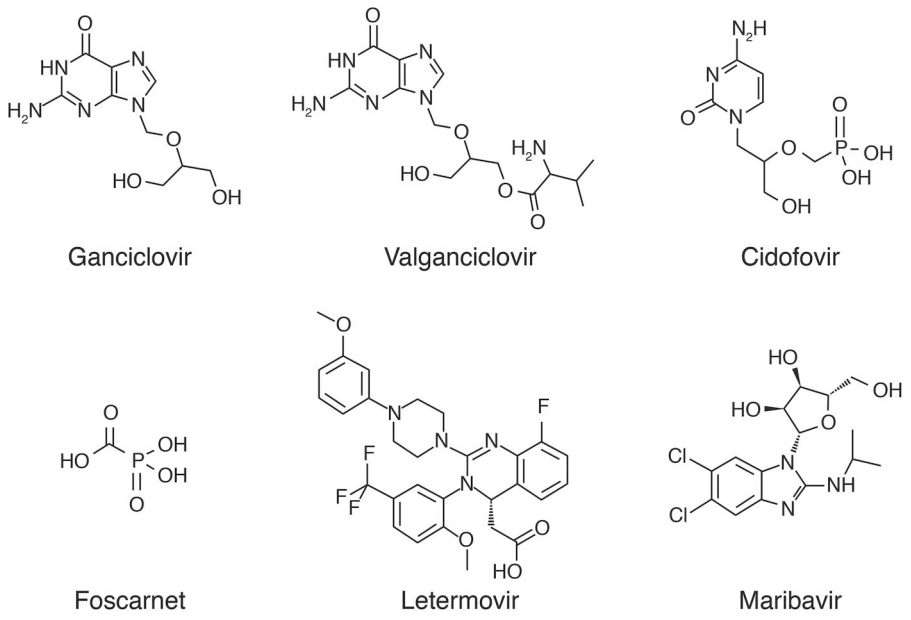

Fig. 1. Chemical structures of antiviral agents that target the viral pUL54 DNA polymerase (i.e., ganciclovir, valganciclovir, cidofovir and foscarnet), the viral terminase complex (i.e., letermovir) and the viral pUL97 kinase (i.e., maribavir) of human cytomegalovirus.

(Andrei et al., 2009). They all target the viral DNA polymerase (pol) (Zarrouk et al., 2017). Ganciclovir (GCV) was the first drug to be approved for this indication and is still the first line treatment for HCMV infections in immunocompromised patients. Following uptake into infected cells, GCV, a nucleoside analogue of guanosine (Fig. 1), is selectively phosphorylated by the HCMV phosphotransferase (the product of the *UL97* gene, pUL97, a serine/threonine kinase). Subsequently, GCV monophosphate is converted by cellular kinases into its active triphosphate form, which acts as a potent inhibitor of the HCMV DNA pol (the product of the *UL54* gene, pUL54) by competing with deoxyguanosine triphosphate on the enzyme binding site (Fig. 2). Ganciclovir triphosphate is a competitive inhibitor of deoxyguanosine triphosphate. Ganciclovir triphosphate is also incorporated into viral DNA where it slows down and eventually stops chain elongation (Biron et al., 1985). The effective concentration of GCV that reduces cytopathic effects induced by HCMV in fibroblast cells by 50% (EC_{50}) is approximately 3.5 ± 2.3 μM (McSharry et al., 2001). GCV can be given orally, intravenously (Cytovene®, Roche) or as an ocular implant (Vitrasert®, Chiron) for the treatment of HCMV retinitis. A prodrug was also developed to increase the poor oral bioavailability (~6%) of GCV. The L-valyl-ester prodrug (Fig. 1), valganciclovir (VGCV, Valcyte®, Roche), exhibits an approximately 10 times improved GCV bioavailability following oral administration compared to the parent compound (Pescovitz et al., 2000). The main toxicity associated with the use of GCV consists of myelosuppression which is an important side effect especially for hematopoietic stem cell transplant (HSCT) recipients.

Two other compounds considered as second line agents are approved for systemic treatment of HCMV infections. Due to their toxicity profiles and the absence of oral formulations, they are usually reserved for patients failing or not tolerating GCV therapy. Cidofovir (CDV, Vistide®, Gilead Sciences) is a non-cyclic analogue of cytidine monophosphate (also called acyclic nucleoside phosphonate; Fig. 1) that only requires activation (phosphorylation) by cellular enzymes to exert its antiviral activity (Cihlar and Chen, 1996). Once in its diphosphate form, CDV inhibits the pUL54 DNA pol by acting as a chain terminator (Fig. 2) (Xiong et al., 1996). Two consecutive incorporations of CDV diphosphate are required to efficiently terminate DNA chain elongation. CDV has a broad spectrum of antiviral activity against most DNA viruses. The EC_{50} value of CDV against HCMV ranges from 0.1 to 0.8 μM. The main side effect of CDV is nephrotoxicity which requires the administration of fluid (hydration) and probenecid to prevent kidney failure. The development of an orally bioavailable ether lipid ester prodrug of CDV (hexadecyloxypropyl-cidofovir), brincidofovir (BCV, Chimerix), has permitted to avoid the dose-limiting renal toxicity of the parent drug and led to a safer alternative for the treatment of drug-resistant HCMV infections in immunocompromised patients (Hostetler, 2010). Treatment with oral BCV (100 mg twice weekly) until week 13 after transplantation significantly reduced the incidence of HCMV infections in HSCT recipients in a phase II study (Marty et al., 2013). Diarrhea was a dose-limiting adverse event in this population when given at 200 mg twice weekly. However, a phase III prophylaxis study with oral BCV (100 mg twice weekly) for 14 weeks after transplantation in HSCT recipients failed to confirm a reduction in HCMV infection through week 24 and was associated with gastrointestinal toxicity (Marty et al., 2019). An intravenous formulation of BCV has been developed to limit the gastrointestinal side effects of the drug. Pharmacokinetic parameters and safety of this formulation are currently evaluated. Foscarnet (FOS, Foscavir®, Astra-Zeneca) is structurally similar to the pyrophosphate anion (Fig. 1). It binds to and blocks the pyrophosphate binding site on the pUL54 DNA pol, thus preventing incorporation of incoming deoxynucleotide triphosphates (dNTPs) into viral DNA (Fig. 2) (Chrisp and Clissold, 1991). FOS has a broad spectrum of antiviral activity against herpesviruses. The EC_{50} value of FOS against HCMV ranges from 30 to 90 μM. The most frequent side effects of FOS are renal impairment and electrolyte abnormalities requiring adequate hydration of the patients and monitoring of serum creatinine levels.

1.2. Mechanism of resistance to approved antiviral agents

Following their identification by genotypic analyses, the role of viral mutations in drug resistance needs to be confirmed by recombinant phenotyping (Chou et al., 2005; Drouot et al., 2013). Briefly, the mutation of interest is transferred, by homologous recombination, in the genome of a baseline laboratory HCMV strain cloned into a bacterial artificial chromosome (BAC) and maintained in bacteria. The recombinant mutant virus is then reconstituted in cultured cells following transfection of the BAC/HCMV clone. The effective drug concentration that reduces the number of viral plaques or the expression of a reporter gene by 50% (EC_{50}) is then determined. The ratio of EC_{50} values of the

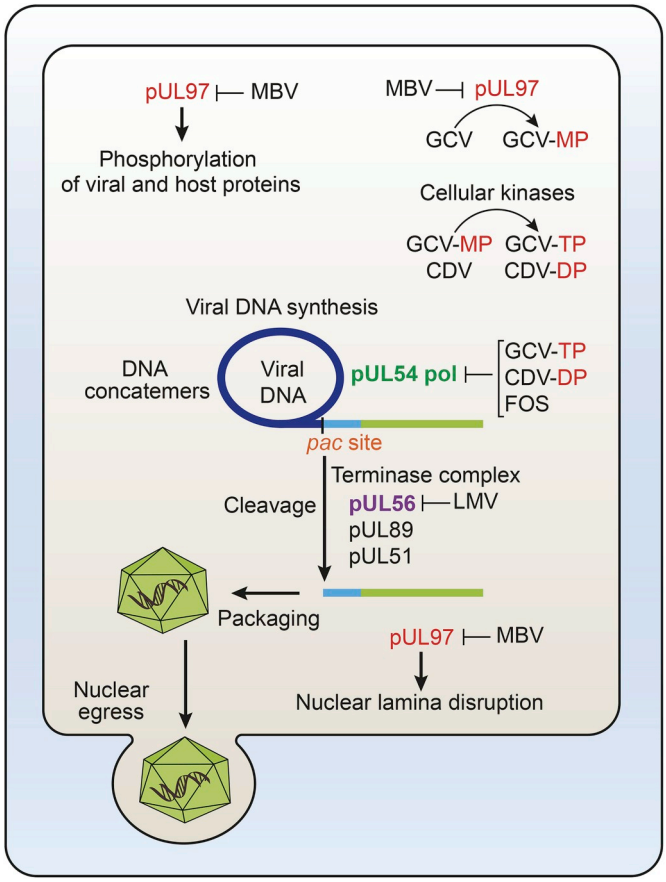

Fig. 2. Mechanism of action of antiviral agents against human cytomegalovirus infection. The viral pUL97 kinase phosphorylates viral and host proteins that are involved in the modulation of cell-cycle to support viral DNA synthesis, in the regulation of viral gene expression, in the promotion of virion morphogenesis and in the induction of nuclear lamina disruption to facilitate nuclear egress of nascent viral particles. Replication of viral DNA by pUL54 DNA polymerase (pol) produces DNA concatemers. The viral terminase complex formed by pUL56, pUL89 and pUL51 subunits is involved in the cleavage of concatemeric DNA at *pac* site and packaging of unit-length genomes into procapsids. On the other hand, the viral pUL97 kinase is also involved in the first phosphorylation step of ganciclovir (GCV) and cellular kinases further phosphorylate GCV monophosphate (GCV-MP) into GCV triphosphate (GCV-TP) which is the active form. Cellular kinases also convert cidofovir (CDV) into active CDV diphosphate (CDV-DP). GCV-TP competes with deoxyguanosine triphosphate on the binding site of pUL54 DNA polymerase. GCV-TP also incorporates into viral DNA where it slows down and eventually stops chain elongation. CDV-DP incorporates into viral DNA, two incorporation of CDV-DP are needed to terminate DNA chain elongation. Foscarnet (FOS) directly inhibits the pUL54 DNA pol activity by competing with pyrophosphate. Letermovir (LMV) interferes with the cleavage and packaging of viral genome by inhibiting the pUL56 subunit of the viral terminase complex. Maribavir (MBV) interferes with the morphogenesis and nuclear egress of nascent viral particles by inhibiting the viral pUL97 kinase activity. The inhibition of pUL97 activity by MBV also prevents the first phosphorylation step of GCV and results in an antagonistic effect against human cytomegalovirus when both drugs are combined.

recombinant mutant virus over the wild-type counterpart is calculated to evaluate the level of drug resistance.

The great majority (> 90%) of drug-resistant HCMV clinical isolates selected from initial treatment with GCV contain one or more mutations in the *UL97* gene whereas mutations in the *UL54* gene are less frequently encountered (Campos et al., 2016; Lurain and Chou, 2010). A594V, L595S, M460V/I, H520Q, C592G and C603W represent the most frequent amino acid substitutions in the pUL97 kinase emerging in GCV-resistant mutants. Other less frequently encountered amino acid changes have been identified at codons 460 and between codons 590 and 607 of the pUL97 kinase. Based on recombinant phenotyping, mutations in *UL97* gene associated with 2- to 5-fold increase in GCV EC_{50} values over the wild-type strain are considered as mediating a low-grade drug resistance whereas mutations associated with 5- to 10-fold increase in GCV EC_{50} values are considered as conferring a moderate level of drug resistance (Kotton et al., 2018). Amino acid substitutions or small deletions in the pUL97 kinase conferring clinical resistance to GCV have no major impact on the viral replicative capacity.

GCV-resistant HCMV clinical isolates with an altered pUL54 DNA pol activity result from numerous amino acid changes widely distributed among the different conserved domains of the enzyme, but mostly occur at codons 395–545 and 809–987. Mutations in *UL54* gene that emerge under GCV therapy can confer cross-resistance to CDV and, less frequently, to FOS. Cross-resistance to GCV and CDV is associated with amino acid substitutions located in the exonuclease domains (codons 301, 408–413 and 501–545) and in region V (codons 981–987) of the enzyme. However, clusters of amino acid changes found in conserved regions II, VI and III are associated with resistance to FOS alone or to both FOS and GCV. Importantly, some mutations have been associated with cross-resistance to all three antivirals. In contrast to pUL97 mutants, recombinant viruses with pUL54 amino acid substitutions conferring drug resistance usually exhibit an attenuated or slow-growth phenotype in cell culture compared to their wild-type counterpart. Combinations of mutations in *UL97* and *UL54* genes multiply the effect of each individual mutation and can result in increased GCV EC_{50} values by > 15-fold and are considered as conferring a high-level of drug resistance (Chou et al., 2007a; Drouot et al., 2014).

Due to the limitations associated with the use of currently-approved antiviral agents, the development of new inhibitors of HCMV infection that act through different mechanisms of action and that exhibit adequate safety profiles is thus an important priority.

2. The viral terminase complex and pUL97 kinase as novel molecular targets

The nuclear phase of HCMV life cycle involves the replication of the viral DNA genome, its cleavage and packaging into procapsids and maturation of DNA-filled capsids. The nucleocapsids are then transported out of the nucleus by egress into the cytoplasm following their envelopment and de-envelopment as they cross the nuclear membrane. The subsequent steps of virion assembly occur in distinct compartments of the cytoplasm. The fully assembled viral particles are finally released from infected cells by an exocytic pathway or after cell lysis. The search for novel inhibitors of HCMV infection has resulted in the discovery of new molecular targets (Britt and Prichard, 2018), especially the viral terminase complex (Gentry et al., 2019; Ligat et al., 2018) and the viral pUL97 kinase (Prichard, 2009). The viral terminase complex is involved in the cleavage and packaging of the viral genome into the capsid whereas the viral pUL97 kinase phosphorylates viral and cellular proteins that have important functions during the viral replication cycle (Fig. 2).

Three chemical classes of derivatives targeting the viral terminase complex were developed to date. The first series of compounds that were shown to target the HCMV terminase complex consists in benzimidazole D-riboside derivatives such as BDCRB (β-D-ribofuranoside-2-bromo-5,6-dichlorobenzimidazole) and GW275175X (a more metabolically stable analogue of BDCRB) (Underwood et al., 1998, 2004). The second class of viral terminase complex inhibitors are phenylenediamine sulphonamide derivatives such as BAY 38–4766 also known as tomeglovir which demonstrated a broader spectrum of activity compared to BDCRB (Reefschlaeger et al., 2001). The lead compound of the third chemical class of viral terminase inhibitors, the quinazolines, is AIC246 (3,4-dihydro-quinazoline-4-yl-acetic acid; Fig. 1) also known as MK-8228 or letermovir (Lischka et al., 2010). The search for more

stable derivatives of BDCRB also led to the discovery of benzimidazole L-ribosides, such as 1263W94 (1H-β-L-ribofuranoside-2-isopropylamino-5,6-dichlorobenzimidazole; Fig. 1) also known as SHP620 or maribavir, which exhibits a novel mechanism of action by targeting the viral pUL97 kinase (Biron et al., 2002). Furthermore, cyclopropavir ((Z)-9-[[2,2-bis-(hydroxymethyl)cyclopropylidene]methyl]guanine) also known as MBX-400 or filociclovir which belongs to a new chemical class, the methylenecyclopropane nucleoside analogues, was shown to be a dual inhibitor of the viral pUL54 DNA pol and pUL97 kinase activities (Chou et al., 2012b; James et al., 2011).

The rapid degradation of BDCRB during pre-systemic metabolism precluded further clinical development. A phase I single escalating dose study showed good safety, tolerability and pharmacokinetics profiles for GW275175X. Single oral doses of up to 2000 mg BAY 38–4766 were safe and well tolerated in healthy male volunteers (Nagelschmitz et al., 1999). However, both GW275175X and BAY 38–4766 were not evaluated beyond phase I clinical trials. Two phase I safety and pharmacokinetic studies with cyclopropavir (CPV) have been performed in normal volunteers (ClinicalTrials.gov Identifiers: NCT02454699 and NCT01433835). More interestingly, letermovir (LMV) has been recently approved under the trade name of Prevymis™ (Merck Sharp & Dohme Corp.) by the US Food and Drug Administration (as of November, 2017) and the European Commission (as of January, 2018) for prophylaxis of HCMV infection in adult HCMV-seropositive recipients of an allogeneic HSCT. Two phase III trials evaluating maribavir (MBV; Shire) treatment of HCMV infection in transplant recipients are ongoing. This review will thus focus on the most advanced compounds, letermovir and maribavir.

3. Antiviral agents targeting the viral terminase complex

3.1. Mechanism of action of letermovir

The replication of HCMV genome produces DNA concatemers composed of head-to-tail viral genomes (Fig. 2). The viral terminase complex is involved in the cleavage of concatemeric viral DNA and the packaging of unit length linear genomes into preformed capsids. The HCMV terminase is a heterotrimeric complex formed by pUL56, pUL89 and pUL51 proteins (Borst et al., 2013). The pUL56 subunit binds to the pUL104 protein that forms the portal through which the viral genome enters the capsid (Dittmer et al., 2005) as well as to the packaging signal (or *pac* site) on the concatemeric viral DNA (Bogner et al., 1998). The pUL56 subunit exhibits an ATPase activity (Hwang and Bogner, 2002; Scholz et al., 2003) that probably provides the energy required to translocate the viral genome into the capsid and to mediate genome cleavage by the pUL89 subunit (Scheffczik et al., 2002). The ATPase activity of pUL56 is increased by up to 30% when it is associated with pUL89 (Hwang and Bogner, 2002). The pUL51 protein represents the smallest component of the HCMV terminase complex (Borst et al., 2013). It was proposed that the mutual interactions of the three subunits regulate the stability, subcellular localization and assembly of the functional terminase complex (Neuber et al., 2017). Additional proteins such as pUL52, pUL77 and pUL93 contribute to the DNA cleavage/packaging process during the HCMV replication cycle but their functions are poorly characterized (Borst et al., 2008, 2016; Koppen-Rung et al., 2016).

LMV inhibits the cleavage of viral DNA concatemers and the formation of mature HCMV virions by targeting the pUL56 subunit of the viral terminase complex (Fig. 2) (Goldner et al., 2011). The antiviral activity of LMV is highly specific to HCMV with effective concentrations in the low nanomolar range and a selectivity index superior to 15,000 (Lischka et al., 2010; Marschall et al., 2012). LMV has no significant antiviral activity against other herpesviruses or non-human CMV strains (Marschall et al., 2012). LMV is approximately 1000-fold more potent than GCV against HCMV and is also effective against viral isolates resistant to currently-available drugs (Marschall et al., 2012). Homologues of proteins that composed the terminase complex have been identified in bacteriophages (except for pUL51) and herpesviruses and have no counterpart in mammalian cells. It is thus expected that LMV would demonstrate a good safety profile. Combinations of LMV with currently-available antiviral drugs resulted in additive effects (with GCV and CDV) and additive/minor antagonistic effects (with FOS) against HCMV in cell culture systems (Wildum et al., 2015). A moderate synergistic effect of LMV with artesunate against HCMV has also been reported *in vitro* (Drouot et al., 2016). These studies suggest that the use of combinations of LMV with other antiviral agents or artesunate could be an interesting strategy for the treatment of HCMV infections.

3.2. Pharmacokinetic studies with letermovir

LMV can be administered orally and intravenously. The intravenous hydroxypropyl β-cyclodextrin formulation of LMV is intended to be used immediately after transplantation as well as in patients presenting gastrointestinal complications that make the ingestion and absorption of oral drugs difficult (Erb-Zohar et al., 2017). Phase I studies conducted in healthy subjects showed that LMV is safe and well tolerated and has a good oral bioavailability (35%). LMV exhibits a safety profile which may be compatible with pre-engraftment prophylaxis in HSCT recipients. Maximum plasma concentrations (C_{max}) of LMV are achieved approximately 1.5 h after oral administration. LMV has a relatively long half-life ($t_{1/2}$) in plasma of approximately 10 h suggesting that once daily dosing is feasible. LMV is highly bound to protein and it is estimated that the percentages of drug bound to plasma proteins are 99% in dog, 98% in mouse and 99% in human. LMV is eliminated primarily unchanged via biliary excretion into feces and excretion into urine is negligible. It was reported that impaired kidney function can affect biliary excretion of drugs (Yeung et al., 2014). LMV exposure is increased in patients with renal impairment compared to healthy individuals (Kropeit et al., 2017b). However, there is no recommendation to adjust LMV dosage in case of creatinine clearance higher than 10 mL/ min. Following multiple oral daily dosing, exposure to LMV appears to be increased in patients with moderate (< 2-fold) and severe (4-fold) hepatic impairment compared to healthy subjects (Kropeit et al., 2017a). LMV demonstrates a weak to moderate inhibitory effect on cytochrome P450 (CYP) activity and was shown to increase exposure to tacrolimus and cyclosporine A which are often administered in immunocompromised patients (Kropeit et al., 2018). On the other hand, cyclosporine A increases the C_{max} and area under the curve (AUC) of LMV in a dose-dependent manner but not tacrolimus. This suggests that the once daily dose of LMV should be reduced (240 mg *versus* 480 mg) when cyclosporine A is co-administered as immunosuppressive therapy. LMV does not affect the pharmacokinetics of posaconazole (a substrate of glucuronosyltransferase and P-glycoprotein) but decreases exposure to voriconazole (a substrate of CYP 2C9/19) suggesting that monitoring of voriconazole effectiveness is recommended when it is co-administered with LMV (Marshall et al., 2018). The most common adverse events reported with the use of LMV are nausea, diarrhea, vomiting, peripheral edema, cough, headache, fatigue and abdominal pain.

3.3. Clinical efficacy studies with letermovir

A phase IIa study evaluated the efficacy, safety and limited pharmacokinetic parameters of oral LMV (40 mg twice daily or 80 mg once daily) or local standard of care (SOC) administered for 14 days as a preemptive treatment of HCMV infection in kidney and kidney/pancreas transplant recipients (Stoelben et al., 2014). Viral clearance was observed in 6 of 12 patients (50%) in the LMV groups compared to 2 of 7 patients (29%) in the SOC group on day 15 of treatment. Three patients in the LMV groups were infected with viral isolates harboring mutations in *UL97* gene or in both *UL97* and *UL54* genes. All three patients responded to LMV treatment. A phase IIb dose-range-finding prophylaxis study evaluated the efficacy of oral daily doses of LMV (60 mg, 120 mg or 240 mg) given for 12 weeks after engraftment in HCMV-seropositive

recipients of allogeneic HSCT (Chemaly et al., 2014). The incidence of all-cause prophylaxis failure was significantly lower in the groups who had received LMV at daily doses of 120 mg (32%) or 240 mg (29%) than in the placebo arm (64%). The incidence of virologic failure was also reduced in the 240 mg dose group (6%) compared to groups who had received 120 mg (19%) or 60 mg (21%) of LMV or the placebo (36%). In addition, LMV at a daily dose of 240 mg significantly reduced the time to onset of prophylaxis failure. This study suggests that the lowest doses of LMV, particularly 60 mg per day, are suboptimal for prophylaxis whereas a dose of 240 mg per day appears to achieve complete suppression of viremia and is well tolerated. A phase III prophylaxis study evaluated the efficacy of oral and intravenous daily doses of LMV (480 mg or 240 mg administered with cyclosporine A) in HSCT recipients seropositive for HCMV (Marty et al., 2017). LMV prophylaxis was initiated at a median of 9 days after transplantation through week 24. The percentage of patients who developed clinically significant HCMV infection was significantly lower in LMV groups (38%) than in the placebo arm (61%) by week 24. All-cause mortality at week 24 post-transplantation was lower in the LMV groups (10%) compared to the placebo arm (16%). A systematic review and meta-analysis compared the safety and efficacy of different interventions (acyclovir, GCV, MBV, BCV, LMV, valacyclovir and a vaccine) for HCMV prophylaxis in allogeneic HSCT recipients (Gagelmann et al., 2018). This study concluded that prophylaxis with GCV and LMV showed the best relative efficacy against HCMV disease and infection. LMV also appears to be the best option as a prophylactic strategy in terms of safety. The recommended prophylactic dose of LMV is 480 mg once daily.

Although LMV has been approved for primary HCMV prophylaxis in HSCT recipients, its efficacy for the treatment of HCMV disease or secondary prophylaxis has been evaluated off-label in case series. The first case report was a lung transplant recipient with disseminated multidrug-resistant HCMV disease who has been successfully treated with low doses of LMV (from 120 mg to 240 mg once daily) but immunosuppressive therapy was also markedly reduced (Kaul et al., 2011). It was then reported that an oral daily dose of 480 mg LMV was successfully used as secondary prophylaxis for GCV-resistant HCMV syndrome in a heart transplant recipient (Chong et al., 2018). In a case series, four solid organ transplant (SOT) recipients received LMV (720 mg daily to 960 mg daily due to lack of effect in one case) as salvage therapy for drug-resistant HCMV retinitis. Despite clinical improvement in all patients, 3 of them were virologically unsuppressed and required alternative treatments (Turner et al., 2019). However, clinical studies involving a large number of patients and investigating the safety, dosage and rate of emergence of drug resistance are required to delineate the optimal use of LMV in the treatment of HCMV infections including those that are refractory or resistant to currently-available drugs.

3.4. Mechanisms of resistance to letermovir

In vitro studies have identified LMV resistance mutations mapping primarily to the *UL56* gene and more rarely to the *UL89* and *UL51* genes encoding the three subunits of the HCMV terminase complex. pUL56 is an 850 amino acid protein encoded by the *UL56* gene of HCMV and conserved among herpesviruses. Twelve conserved regions (numbered I to XII) have been identified in pUL56 and homologous proteins from other herpesviruses (Champier et al., 2008). Two variable regions are located between conserved regions VI and VII and after conserved region XII. The pUL56 subunit exhibits a low natural polymorphism among susceptible and drug-resistant clinical HCMV isolates (inter-strain identity > 97.7% at both nucleotide and amino acid levels) (Pilorge et al., 2014). Mutations conferring resistance to LMV result in amino acid substitutions and are located at codon 25 and between codons 229 and 369 of the pUL56 subunit (Fig. 3A and Table 1). Mutants resistant to LMV were isolated from the HCMV strain AD169 following one-step selection with 10-times the drug EC_{50} or successive passages with increasing drug concentrations (Goldner et al., 2011, 2014). These strains harbored V231L, V236M, L241P, C325Y, R369G, R369M and R369S substitutions in the pUL56 subunit which conferred 5-, 45-, 160-, 8796-, 44-, 13- and 38-fold increase in LMV EC_{50} values. A pUL54 DNA pol exonuclease domain II D413A mutant isolated from a clinical specimen was shown to exhibit an enhanced DNA replication error rate (Marfori et al., 2007). Additional mutations were selected after exposure of a similar error-prone exonuclease mutant to LMV (Chou, 2015, 2017a). These mutations conferred various levels of resistance to the drug, i.e., less than 5-fold increase in EC_{50} values for V231A, T244K, L257I, F261C, F261L, Y321C and M329T mutants; between 5- and 15-fold increase in EC_{50} values for N232Y, E237D and K258E mutants and more than 1000-fold increase in EC_{50} values for C325F and C325R mutants. Some mutations were also selected from a BAC clone of HCMV strain AD169 expressing a secreted alkaline phosphatase (SEAP) reporter gene and exposed to LMV (Chou, 2017b; Chou et al., 2018b). These mutations were also shown to confer various levels of resistance to LMV, i.e., less than 5-fold increase in EC_{50} values for S229F, V236A, L254F, L328V, A365S and N368D mutants; between 5- and 15-fold increase in EC_{50} values for C25F, V236L and L257F mutants and more than 1000-fold increase in EC_{50} value for C325W mutant. A365S, N368D, R369G, R369M and R369S substitutions are located in conserved region VI of the pUL56 subunit. Mutations in the *UL56* gene conferring resistance to LMV have minimal to low impact on viral growth fitness compared to their wild-type counterparts. Combinations of two or three mutations in the *UL56* gene result in markedly increased levels of resistance to LMV (Chou, 2015, 2017b; Chou et al., 2018b).

pUL89 is an 674 amino acid protein encoded by the *UL89* gene of HCMV and conserved among herpesviruses. Twelve conserved regions (numbered I to XII) were identified in pUL89 subunit and its counterpart in other herpesviruses (Champier et al., 2007). The four amino acid motifs [i.e., adenine binding site, Walker A or motif I (γ-phosphate sensor), Walker B or motif II (ATPase motor) and motif III (ATPase coupling helicase)] identified in bacteriophage T4 (Mitchell et al., 2002) are located in conserved regions II, III and V (for the last two motifs), respectively. The natural polymorphism of the pUL89 subunit was shown to be low in susceptible and drug-resistant HCMV clinical isolates (Pilorge et al., 2014). Amino acid changes in the pUL89 subunit of the viral terminase complex conferring resistance to LMV are reported in Fig. 3B and Table 2. Exposure of an error-prone exonuclease HCMV mutants to different inhibitors of the viral terminase complex (LMV, GW275175X or tomeglovir) allowed the identification of mutations conferring resistance to LMV in the *UL89* gene (Chou, 2017a,b). All these amino acid changes (N320H, N329S, D344E and T350M) are located in conserved region V of the pUL89 subunit and confer low-grade resistance to LMV. Recombinant viruses harboring LMV-resistant mutations in the *UL89* gene exhibit normal cytopathic effects and viral growth in cell culture. The addition of the D344E substitution in pUL89 to E237D, F261L, M329T or Q204R substitution in pUL56 increases the EC_{50} values of the single pUL56 mutants.

Finally, a mutation conferring resistance to LMV was identified in the *UL51* gene encoding the third component of the viral terminase complex, pUL51 (Chou, 2017b). pUL51 is an 157 amino acid protein which has no equivalent in bacteriophages and is homologous to pUL33 in herpes simplex virus 1. The sequence similarity between the two proteins is limited and comprises only the C-terminal part (amino acids 73 to 149 of pUL51) whereas the N-terminal part of the protein is not conserved among herpesviruses (Borst et al., 2013). P91S substitution was selected from a BAC clone of HCMV strain AD169 exposed to LMV and was shown to confer low-grade resistance to the drug (Fig. 3C and Table 3). The viral growth of the mutant recombinant virus was not affected compared to the wild-type strain. Combinations of P91S substitution in pUL51 with several substitutions (S229F, V236M and/or R329M) in pUL56 multiply the fold-changes of each individual

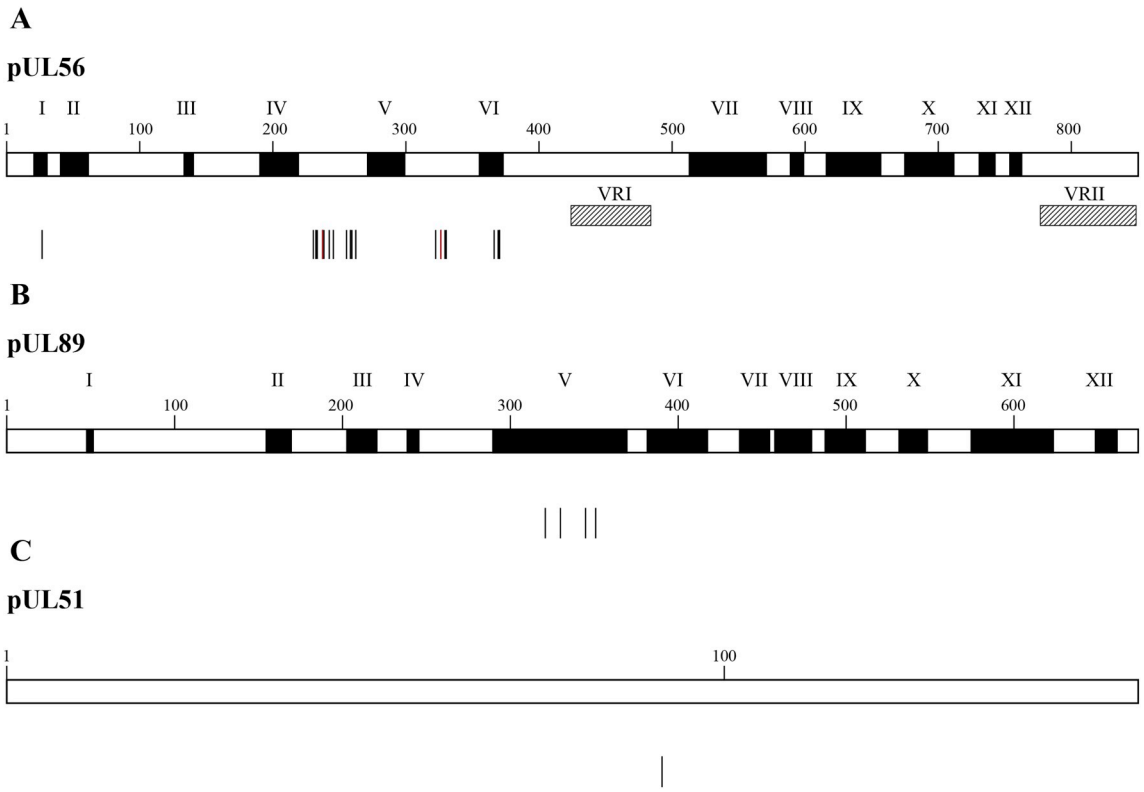

Fig. 3. Amino acid substitutions conferring resistance to letermovir identified in HCMV laboratory strains and clinical specimens that were confirmed by recombinant phenotyping. Panel **A** shows amino acid changes in the pUL56 subunit conferring letermovir resistance. Conserved regions in pUL56 and among herpesviruses are represented by the black boxes and numbered I to XII. Two variable regions are represented by hatched boxes and indicated as VRI and VRII. Panel **B** shows amino acid changes in the pUL89 subunit associated with resistance to letermovir. Conserved regions in pUL89 and among herpesviruses are represented by the black boxes and numbered I to XII. Panel **C** shows amino acid change in pUL51 subunit associated with letermovir resistance. Bars (|) indicate amino acid substitutions conferring letermovir resistance. Bars in red correspond to amino acid changes conferring letermovir resistance detected in clinical specimens. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

substitution whereas combination with a substitution in pUL89 (D344E) is additive.

The first clinical isolate resistant to LMV was detected in a HSCT recipient enrolled in the phase IIb dose-ranging prophylaxis study (Lischka et al., 2016). This patient received a suboptimal dose of 60 mg LMV per day and was considered to have a virologic failure (Chemaly et al., 2014). Genotypic analyses of plasma samples revealed the emergence of a mutation in *UL56* gene resulting in a V236M substitution that was already known as mediating a high-grade resistance to LMV by recombinant phenotyping (Goldner et al., 2014). Following discontinuation of LMV, the patient responded to treatment with VGCV 900 mg twice daily. During the phase III prophylaxis study, genotypic resistance testing of *UL56* and *UL89* genes was performed on plasma samples from HSCT recipients who had received LMV and developed clinically significant HCMV infection (Marty et al., 2017). Mutations in the *UL56* gene resulting in V236M and C325W substitutions were identified in clinical specimens of two patients (Merck Sharp and Dohme Corp, 2017). The C325W substitution was already confirmed as conferring a high-grade resistance to LMV (Chou et al., 2018b). Recently, a HSCT patient on LMV prophylaxis had a HCMV pneumonia breakthrough (Knoll et al., 2018). Genotypic analysis of plasma samples detected a mutation in *UL56* gene resulting in a C325F substitution. This mutation was already shown to confer high-level of resistance to LMV by recombinant phenotyping (Chou, 2015). LMV was discontinued and alternative therapies were initiated.

A lung transplant recipient who had developed a drug-resistant HCMV infection was treated with LMV. Following virologic failure, genotypic testing identified a mutation in the *UL56* gene resulting in a C325Y substitution (Cherrier et al., 2018). This mutation was already confirmed as conferring a high-level resistance to LMV by recombinant phenotyping (Chou, 2015; Chou et al., 2018b; Goldner et al., 2014). This patient was then successfully treated with FOS. Furthermore, genotypic analyses were performed on plasma samples from 3 of 4 patients from a case series who had received salvage therapy with LMV for drug-resistant HCMV infection and who were virologically unsuppressed (Turner et al., 2019). The analyses revealed that 2 of 3 clinical specimens respectively harbored C325F and C325Y substitutions in pUL56. The three patients achieved virologic suppression after switching to alternative therapy with VGCV or FOS. By using an error-prone exonuclease HCMV variant, mutations conferring drug resistance selected *in vitro* appeared earlier for LMV (median of 3 passages) than for FOS (median of 15 passages) and resulted in minimal alteration of viral growth (Chou, 2015). These data suggest that LMV may have a low viral genetic barrier to resistance. Thus, the off-label use of LMV for salvage therapy of refractory or drug-resistant HCMV infections should be done with caution and monitoring of LMV resistance should be performed early after viral rebound. A larger phase II study of letermovir treatment for patients experiencing refractory or resistant cytomegalovirus infection or disease with concurrent organ dysfunction (ClinicalTrials.Gov Identifier: NCT03728426) is ongoing and should provide useful data in that context. Patients with refractory or resistant HCMV infection will be treated with LMV at a dose of 480 mg (240 mg if administered with cyclosporine) daily for up to 12 weeks (an additional 12 weeks of treatment could be given for secondary prophylaxis if clinically indicated).

Table 1
Amino acid changes in the pUL56 subunit of the terminase complex conferring resistance to letermovir.

pUL56	Origin of strain	LMV EC_{50} ratio (mutant/WT)	Assays	References
C25F	Lab-derived strain[a] (under both LMV and GCV)	5.4	SEAP	Chou et al. (2018b)
S229F	Lab-derived strain[a] Recombinant virus	1.8	SEAP	Chou (2017b)
V231A	Lab-derived strain[b] Recombinant virus	2.1	SEAP	Chou (2015)
V231L	Lab-derived strains[b, c] Recombinant virus	5.0-5.6-8.1-28	PRA, SEAP, fluorescence	(Chou, 2015, 2017b; Chou et al., 2018b; Goldner et al., 2014)
N232Y	Lab-derived strain[b] Recombinant virus	17	SEAP	Chou (2017a)
V236A	Lab-derived strain[a] (under both LMV and GCV)	2.9	SEAP	Chou et al. (2018b)
V236L	Lab-derived strain[b] Recombinant virus	14	SEAP	Chou (2015)
V236M	**Lab-derived strains[b, c] Recombinant virus Clinical isolate**	**19-31-32-45-49**	**PRA, SEAP, fluorescence, GLuc**	(Chou, 2017b; Chou et al., 2018b; Goldner et al., 2014; Lischka et al., 2016; Piret et al., 2017)
E237D	Lab-derived strain[b] Recombinant virus	10–16	SEAP	(Chou, 2015, 2017a)
L241P	Lab-derived strains[b, c] Recombinant virus	96-160-173-200-218-266	PRA, SEAP, fluorescence	(Chou, 2015, 2017b; Goldner et al., 2011, 2014)
T244K	Lab-derived strain[b] Recombinant virus	3.3	SEAP	Chou (2015)
L254F	Lab-derived strain[a] Recombinant virus	3.2	SEAP	Chou (2017b)
L257F	Lab-derived strain[a] Recombinant virus	8.6–14	SEAP	(Chou, 2017b; Chou et al., 2018b)
L257I	Lab-derived strain[b] Recombinant virus	4.9	SEAP	Chou (2015)
K258E	Lab-derived strain[b] Recombinant virus	14	SEAP	Chou (2017a)
F261C	Lab-derived strain[b] Recombinant virus	4.4	SEAP	Chou (2015)
F261L	Lab-derived strain[b] Recombinant virus	2.5–2.8	SEAP	(Chou, 2015, 2017a)
Y321C	Lab-derived strain[b] Recombinant virus	4.6	SEAP	Chou (2015)
C325F	**Lab-derived strain[b] Recombinant virus Clinical isolate**	**> 3000**	**SEAP**	(Chou, 2015; Turner et al., 2019)
C325R	Lab-derived strain[b] Recombinant virus	> 3000	SEAP	Chou (2015)
C325Y	Lab-derived strains[b, c] **Recombinant virus Clinical isolates**	**> 3000-5413-5870-8800**	**PRA, SEAP, fluorescence**	(Cherrier et al., 2018; Chou, 2015; Chou et al., 2018b; Goldner et al., 2014; Turner et al., 2019)
C325W	**Lab-derived strain[a] (under both LMV and GCV) Clinical isolate**	**9300**	**SEAP**	(Chou et al., 2018b; Marty et al., 2017)
L328V	Lab-derived strain[a] (under both LMV and GCV)	1.9	SEAP	Chou et al. (2018b)
M329T	Lab-derived strain[b] Recombinant virus	4.4–4.5	SEAP	(Chou, 2015, 2017a)
A365S	Lab-derived strain[a] (under both LMV and GCV)	2.0	SEAP	Chou et al. (2018b)
N368D	Lab-derived strain[a] Recombinant virus	2.0	SEAP	Chou (2017b)
R369G	Lab-derived strain[c] Recombinant virus	44	PRA, fluorescence	Goldner et al. (2014)
R369M	Lab-derived strains[a, c] Recombinant virus	13-16-17-23	PRA, SEAP, fluorescence	(Chou, 2017b; Goldner et al., 2014)
R369S	Lab-derived strain[c] Recombinant virus	38-48-81	PRA, fluorescence	(Goldner et al., 2011, 2014)
Multiple amino acid changes in pUL56 subunit				
C25F, V231L	Recombinant virus	46	SEAP	Chou et al. (2018b)
T244K, F261L	Recombinant virus	8.2	SEAP	Chou (2015)
E237D, T244K, F261L	Recombinant virus	104	SEAP	Chou (2015)

(*continued on next page*)

Table 1 (*continued*)

pUL56	Origin of strain	LMV EC_{50} ratio (mutant/WT)	Assays	References
V236L, L257I	Recombinant virus	260	SEAP	Chou (2015)
V236M, L257I, M329T	Recombinant virus	> 3000	SEAP	Chou (2015)
S229F, L254F, L257I	Recombinant virus	54	SEAP	Chou (2017b)

LMV, letermovir; WT, wild-type; PRA, plaque reduction assay; SEAP, secreted alkaline phosphatase; GLuc, *Gaussia* luciferase; EC_{50}, concentration of antiviral that reduces the number of plaques or fluorescence or SEAP or GLuc activity by 50%.
Lines in bold show amino acid substitutions identified in clinical specimens.
[a] Bacterial artificial chromosome (BAC) clone of AD169 strain expressing a SEAP reporter gene.
[b] Exonuclease domain II (D413A) DNA polymerase HCMV mutant.
[c] AD169 strain.
Drug-susceptible, EC_{50} ratio < 1.7; Borderline level of resistance, EC_{50} ratio ≥1.7 and ≤ 1.9; Drug-resistant, EC_{50} ratio ≥2.0.

4. Antiviral agents targeting the viral pUL97 kinase

4.1. Mechanism of action of maribavir

The pUL97 is a serine/threonine protein kinase that phosphorylates itself and multiple viral and host proteins that are involved in many steps of the viral replication cycle (Fig. 2) (Michel et al., 1996). pUL97 is associated with pp65 tegument protein in the HCMV virion (Kamil and Coen, 2007). pUL97 is expressed early during infection and localizes predominantly in the nucleus whereas it is observed later in the cytoplasm (Michel et al., 1996). The viral pUL97 kinase was shown to phosphorylate several viral proteins such as the major immediate early promoter (Bigley et al., 2013), the viral polymerase accessory protein pUL44 (Krosky et al., 2003b; Marschall et al., 2003), the nuclear mRNA export factor pUL69 (Thomas et al., 2009) and the viral pp65 tegument protein (Becke et al., 2010). The viral pUL97 kinase is a functional orthologue of cellular cyclin-dependent kinase complexes (CDKs). pUL97 phosphorylates the retinoblastoma tumor suppressor protein (Rb) on sites normally phosphorylated by CDKs. Once phosphorylated, Rb is unable to repress host genes required for cell-cycle progression to S phase that are crucial for HCMV DNA synthesis (Reim et al., 2013). On the other hand, the nuclear egress complex (NEC), composed of the two subunit pUL50 and pUL53, recruits the viral pUL97 kinase to the nuclear rim. pUL97 then phosphorylates lamin A/C (Hamirally et al., 2009) and the NEC leading to disruption of the nuclear lamina and translocation of viral particles into the cytoplasm (Sharma et al., 2015). Thus, the viral pUL97 kinase plays critical roles in cell-cycle modulation to support viral DNA synthesis, in the regulation of viral gene expression, in the promotion of virion morphogenesis and in the induction of nuclear lamina disassembly to facilitate the nuclear egress of nascent viral particles (Prichard, 2009). Autophosphorylation of pUL97 is presumed to be involved in the regulation of its enzymatic activities (He et al., 1997).

MBV was shown to be an inhibitor of the pUL97 kinase activity (Fig. 2) (Biron et al., 2002; Williams et al., 2003). MBV does not require intracellular phosphorylation to be active. In cell culture, MBV is about 10-fold more potent against HCMV than GCV with an EC_{50} value of approximately 0.3 μM. MBV is also effective against HCMV strains resistant to GCV (Drew et al., 2006). Laboratory-engineered pUL97-deficient HCMV mutant exhibits a severe replicative defect compared to the wild-type parental strain highlighting the essential role of this enzyme in the viral replicative cycle (Prichard et al., 1999). The invariant lysine in the kinase active site is located at codon 355. Amino acid deletion or changes at this position such as K355del, K355M or K355Q abolish kinase activity (He et al., 1997; Marschall et al., 2001). Cells infected by pUL97-defective HCMV mutants are characterized by an inefficient viral DNA synthesis and a sequestration of structural proteins, especially the pp65 tegument protein, in nuclear aggregates which results in an alteration of virion morphogenesis. Inhibition of pUL97 kinase activity by MBV leads to defects in the viral replication cycle that correspond to the pUL97-defective phenotype. MBV interferes with the morphogenesis and nuclear egress of nascent viral

Table 2
Amino acid changes in the pUL89 subunit of the terminase complex conferring resistance to letermovir.

pUL89	Origin of strain	LMV EC_{50} ratio (mutant/WT)	Assays	References
N320H	Lab-derived strain[a] Recombinant virus	1.8	SEAP	Chou (2017a)
N329S	Lab-derived strain[a] (under tomeglovir) Recombinant virus	2.0	SEAP	Chou (2017a)
D344E	Lab-derived strain[a] (under GW275175X) Recombinant virus	1.6–1.8	SEAP	(Chou, 2017a, b)
T350M	Lab-derived strain[a] (under tomeglovir) Recombinant virus	2.8	SEAP	Chou (2017a)
Amino acid changes in pUL56 and pUL89 subunits				
E237D (pUL56) and D344E (pUL89)	Lab-derived strain[a] Recombinant virus	34	SEAP	Chou (2017a)
F261L (pUL56) and D344E (pUL89)	Lab-derived strain[a] Recombinant virus	4.8	SEAP	Chou (2017a)
M329T (pUL56) and D344E (pUL89)	Lab-derived strain[a] Recombinant virus	8.0	SEAP	Chou (2017a)
Q204R (pUL56) and D344E (pUL89)	Lab-derived strain[a] (under GW275175X) Recombinant virus	2.5	SEAP	Chou (2017a)

LMV, letermovir; WT, wild-type; SEAP, secreted alkaline phosphatase; EC_{50}, concentration of antiviral that reduces the SEAP activity by 50%.
[a] Exonuclease domain II (D413A) DNA polymerase HCMV mutant.
Drug-susceptible, EC_{50} ratio < 1.7; Borderline level of resistance, EC_{50} ratio ≥1.7 and ≤ 1.9; Drug-resistant, EC_{50} ratio ≥2.0.

Table 3
Amino acid changes in the pUL51 subunit of the terminase complex conferring resistance to letermovir.

pUL51	Origin of strain	LMV EC_{50} ratio (mutant/WT)	Assays	References
P91S	Lab-derived strain[a] Recombinant virus	2.1	SEAP	Chou (2017b)
Amino acid changes in pUL51 and pUL56 subunits				
P91S (pUL51) and S229F (pUL56)	Recombinant virus	6.3	SEAP	Chou (2017b)
P91S (pUL51) and V236M (pUL56)	Recombinant virus	28	SEAP	Chou (2017b)
P91S (pUL51) and R369M (pUL56)	Recombinant virus	126	SEAP	Chou (2017b)
P91S (pUL51) and S229F, L254F, L257I (pUL56)	Recombinant virus	290	SEAP	Chou (2017b)
Amino acid changes in pUL51 and pUL89 subunits				
P91S (pUL51) and D344E (pUL89)	Recombinant virus	4.0	SEAP	Chou (2017b)

LMV, letermovir; WT, wild-type; SEAP, secreted alkaline phosphatase; EC_{50}, concentration of antiviral that reduces the SEAP activity by 50%.
[a] Bacterial artificial chromosome (BAC) clone of AD169 strain expressing a SEAP reporter gene.
Drug-susceptible, EC_{50} ratio < 1.7; Borderline level of resistance, EC_{50} ratio ≥1.7 and ≤ 1.9; Drug-resistant, EC_{50} ratio ≥2.0.

particles in infected cells (Krosky et al., 2003a). Enzyme kinetic experiments demonstrated that MBV is a competitive inhibitor of adenosine triphosphate (ATP) binding to the pUL97 kinase (Shannon-Lowe and Emery, 2010). The HCMV pUL97 kinase does not appear to phosphorylate natural deoxynucleosides but it is implicated in the phosphorylation of GCV (Michel et al., 1996). Thus, as MBV inhibits pUL97 kinase activity, it is expected to interfere with the phosphorylation of GCV and lead to an antagonistic effect against HCMV infection if both drugs are co-administered (Chou and Marousek, 2006). Combinations of MBV with FOS, CDV and LMV showed synergistic effects against wild-type and drug-resistant HCMV strains whereas combinations of MBV with rapamycin were strongly synergistic (Chou et al., 2018a; O'Brien et al., 2018). These data suggest that the benefit of combining MBV with other antiviral agents or rapamycin should be further investigated. MBV demonstrated low toxicity against bone marrow progenitors and different human leukemia cell lines *in vitro* (Biron et al., 2002; Chan et al., 2000). In contrast to GCV, the lack of toxicity of MBV for the bone marrow could allow an initiation of drug prophylaxis even before engraftment in HSCT recipients.

4.2. Pharmacokinetic studies with maribavir

Preclinical safety and pharmacokinetic studies demonstrated that MBV has a favorable safety profile, good oral bioavailability and lower toxicity than currently-available drugs (Koszalka et al., 2002). The mean oral bioavailability of MBV is greater than 90% in rat and approximately 50% in monkey. MBV is eliminated primarily via biliary excretion in rat and monkey whereas renal and metabolic clearance are minor elimination pathways. MBV is highly bound to plasma proteins *in vitro*, primarily to albumin. Protein binding of MBV is estimated to be 98% in human, 84% in monkey, 88% in rat and 85% in mouse.

Two phase I clinical trials evaluated the safety and pharmacokinetics of single escalating oral doses of MBV (from 50 mg to 1600 mg) in healthy and human immunodeficiency virus (HIV)-infected subjects (Wang et al., 2003). At least 30%–40% of an oral dose of MBV is absorbed with a C_{max} reached within 1–3 h after administration. MBV is rapidly eliminated with a mean $t_{1/2}$ in plasma of 3–5 h. MBV is extensively metabolized and less than 2% of the drug is excreted unchanged in urine. Following oral administration of 400 mg MBV twice daily, the steady-state unbound drug concentration in plasma would be maintained above the *in vitro* EC_{50} determined by DNA hybridization over the entire dosing interval. After a single oral dose of 400 mg MBV, no statistically significant differences in drug pharmacokinetics based on total and unbound plasma concentrations were observed between subjects with normal renal function and subjects with mild/moderate or severe renal impairment (Swan et al., 2007). Multiple oral doses of MBV did not affect CYP 1A2, CYP 2C9, CYP 3A, N-acetyltransferase-2 and xanthine oxydase activities in healthy adults (Ma et al., 2006). MBV dosage does not need to be adjusted when it is co-administered with inhibitors or substrates of CYP 3A4 since it is estimated that only one third of a MBV dose is cleared by this pathway (Goldwater et al., 2008). Co-administration of 400 mg MBV twice daily with tacrolimus increased exposure to tacrolimus and most likely results from MBV-induced inhibition of CYP 3A4, P-glycoprotein or both (Pescovitz et al., 2009). MBV was also shown to increase tacrolimus or sirolimus levels in 10% of treated patients (Papanicolaou et al., 2018). This suggests that monitoring of immunosuppressor concentrations in patients treated with MBV should be required. The adverse events related to the use of MBV include most frequently taste disturbance, nausea, vomiting, diarrhea and headache.

4.3. Clinical efficacy studies with maribavir

A phase I study with multiple oral doses of MBV (100 mg, 200 mg or 400 mg three times a day or 600 mg, 900 mg or 1200 mg twice a day) for 28 days in HIV-infected men with asymptomatic HCMV shedding demonstrated that the drug has a good oral bioavailability and is generally well tolerated (Lalezari et al., 2002). Moreover, MBV reduced the viral shedding in semen with a median decrease in HCMV titers of 2.9–3.7 log PFUs/mL at all tested dosages. Prophylaxis with MBV (100 mg twice daily, 400 mg once daily or 400 mg twice daily) was evaluated in allogeneic HSCT recipients seropositive for HCMV (Winston et al., 2008). Within the first 100 days after transplantation, the incidence of HCMV infection based on pp65 antigenemia was lower in the three MBV dose groups (15%, 19% and 15%) compared to placebo arm (39%). A reduction in the incidence of HCMV infection was also observed based on plasma HCMV DNA in the three MBV groups (7%, 11% and 19%) compared to placebo (46%). The lowest dose of MBV (100 mg twice daily) appeared to be as effective as the higher doses for prevention of HCMV infection. Pharmacokinetic analysis showed that 100 mg MBV twice daily and 400 mg MBV once daily provided similar trough plasma concentrations whereas 400 mg MBV twice daily resulted in higher C_{max} and AUC with no improved antiviral activity but more frequent adverse events. A first phase III study evaluated MBV prophylaxis at a dose of 100 mg twice daily in HCMV-seropositive recipients of allogeneic HSCT from seropositive donors (Marty et al., 2011). The incidence of HCMV infection was reduced in the MBV group (26%) compared to placebo (35%) based on pp65 antigenemia within the first 100 days after transplantation but not based on plasma viral DNA (28% *versus* 30%). A second phase III study compared the efficacy of prophylactic oral MBV (100 mg twice daily) and oral GCV (1000 mg three times daily) for prevention of HCMV disease in seronegative recipients of seropositive liver transplants (Winston et al., 2012). However, this study failed to demonstrate the non-inferiority of oral MBV (12%) to oral GCV (8%) for prevention of HCMV disease. It was proposed that the cohort size of the phase II study was too small to evaluate statistical differences in efficacy between MBV dose groups (Marty and Boeckh, 2011). The lowest dose of MBV (100 mg twice daily) that was selected from the phase II study was probably insufficient and resulted in a lack of drug efficacy in the two phase III

studies. Furthermore, MBV prophylaxis in the phase III study could have been initiated too late, i.e., at a median time of 24 days after transplantation which is generally after engraftment. Ten percent of HSCT recipients screened for enrolment had HCMV infection before engraftment and were excluded from the study to increase the safety of patients assigned to the placebo group. These higher risk patients might have benefited the most from MBV prophylaxis. However, case reports of salvage therapy with MBV at higher doses (up to 800 mg twice daily) for a median of 207 days in 6 patients (5 SOT recipients and 1 HSCT recipient) with refractory or drug-resistant HCMV infections suggested potential therapeutic utility of the drug (Avery et al., 2010). Adequate clinical and virological responses were also reported in half of a series of 12 patients (3 bone marrow transplant recipients and 9 SOT recipients) infected with drug-resistant HCMV strains and treated with at least 800 mg of MBV daily (Alain et al., 2013). Two phase II, dose-ranging, efficacy studies were then performed to evaluate the efficacy of higher doses of MBV. The first study compared the safety, tolerability and efficacy of pre-emptive MBV (400 mg, 800 mg or 1200 mg twice daily) and VGCV (900 mg twice daily) administered for up to 12 weeks in HSCT and SOT recipients. In all dose groups, MBV (77%) had similar efficacy as VGCV (65%) for clearing HCMV viremia within 6 weeks of treatment with a similar effect between MBV doses (78%, 83% and 72% of patients in the 400 mg, 800 mg and 1200 mg dose groups, respectively) (Maertens et al., 2016). The second phase II study evaluated the safety, tolerability and efficacy of escalating doses of MBV (400 mg, 800 mg or 1200 mg twice daily) administered for up to 24 weeks for the treatment of refractory or drug-resistant HCMV disease in transplant recipients. In all MBV dose groups, 67% of patients had no detectable levels of viral DNA in plasma within 6 weeks of treatment with similar effects observed between the different MBV doses (70%, 63% and 67% of patients in the 400 mg, 800 mg and 1200 mg dose groups, respectively) (Papanicolaou et al., 2018). Two phase III studies with MBV are ongoing. The first one compares the efficacy and safety of MBV (400 mg twice daily) and VGCV (900 mg twice daily) treatment of HCMV infections in HSCT recipients (ClinicalTrials.gov Identifier: NCT02927067). The second phase III study compares the efficacy and safety of MBV (400 mg twice daily) with investigator-assigned drug for the treatment of refractory or drug-resistant HCMV infections in transplant recipients (ClinicalTrials.gov Identifier: NCT02931539).

4.4. Mechanism of resistance to maribavir

In vitro studies have identified MBV resistance mutations mapping primarily to the *UL97* gene as well as compensatory mutations in the *UL27* gene. The pUL97 kinase is conserved among members of the herpesvirus family. The catalytic domain of protein kinases consists of eleven major conserved regions numbered I to XI, with region I having the highest level of homology (Hanks et al., 1988). The viral pUL97 kinase is an 707 amino acid protein. The ATP-binding site, the phosphate transfer domain (P-loop) and the substrate-recognition site (catalytic loop) correspond to codon ranges located at positions 337–345 (region I), 481–483 (region VII) and 574–579 (region IX), respectively. Amino acid changes or deletions in the pUL97 kinase conferring resistance to MBV are reported in Fig. 4A, Table 4. The first mutation conferring resistance to MBV (resulting in L397R substitution) selected in a laboratory-derived strain mapped to the *UL97* gene and allowed to establish the mode of action of the drug (Biron et al., 2002). This mutation confer high-grade resistance to MBV (i.e., > 200-fold) but remained susceptible to currently-available drugs. Clinical HCMV isolates exposed to MBV *in vitro* allowed the selection of V353A and T409M substitutions that confer 15- and 81-fold increases in EC_{50} values (Chou et al., 2007b). Exposure of an error-prone exonuclease HCMV mutant to MBV accelerated the selection of mutations in *UL97* gene including the three already known mutations and emphasized their relative frequencies. New amino acid changes at codon 411 were also selected such as H411L, H411N and H411Y that confer 69-, 9- and 12-fold increases in EC_{50} values (Chou and Marousek, 2008). Exposure of the exonuclease HCMV mutant to higher MBV concentrations selected combinations of substitutions in the pUL97 kinase such as V353A/H411L and V353A/H411Y that confer high-levels of drug resistance (227- and 164-fold increase in EC_{50} values, respectively). All these amino acid changes are located in the vicinity of the ATP-binding domain (codons 353, 397, 409 and 411) and did not overlap with those conferring resistance to GCV. These HCMV variants were thus susceptible to GCV. The error-prone exonuclease HCMV mutant exposed to MBV also allowed the selection of the L337M substitution that is located in a conserved region, the ATP-binding (P-loop) and confers 3.5-fold increase in EC_{50} values (Chou et al., 2012a). Other amino acid changes, F342S and V356G, selected under CPV are also located in the P-loop and exhibit a

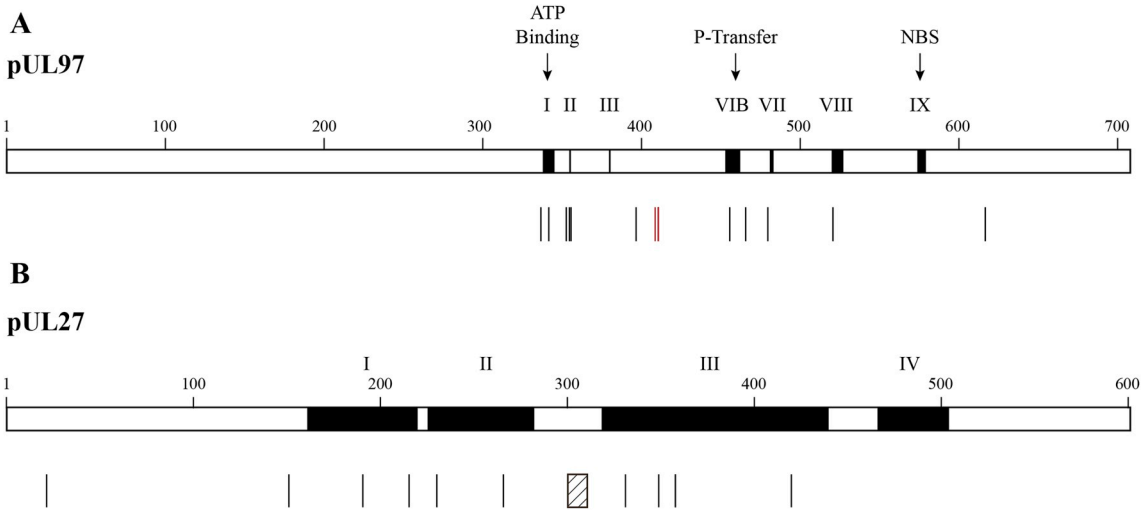

Fig. 4. Amino acid substitutions conferring resistance to maribavir identified in HCMV laboratory strains and clinical specimens that were confirmed by recombinant phenotyping. Panel **A** shows amino acid changes in the pUL97 kinase associated with maribavir resistance. The ATP-binding site, the phosphate transfer (P-transfer) domain, the nucleoside-binding site (NBS) and some regions conserved among the protein kinase family (i.e., I, II, III, VIB, VII, VIII and IX) are represented by the black boxes. Panel **B** shows amino acid changes in the pUL27 conferring resistance to maribavir. Conserved regions in pUL27 and among herpesviruses are represented by the black boxes and numbered I to IV. Bars (|) indicate amino acid substitutions and hatched box represents deletion of codons 301–311 associated with maribavir resistance. Bars in red correspond to amino acid changes conferring maribavir resistance detected in clinical specimens. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Amino acid changes in the pUL97 kinase conferring resistance to maribavir.

pUL97	Origin of strain	MBV EC_{50} ratio (mutant/WT)	GCV EC_{50} ratio (mutant/WT)	Assays	References
L337M	Lab-derived strain[a] Recombinant virus	3.4–3.5	1.02	SEAP	Chou et al. (2012a)
F342S	Lab-derived strain[a] (under CPV) Recombinant virus	18	7.8	SEAP	Chou et al. (2013)
V353A	Lab-derived strains[a,b] Recombinant virus	10-12-15-16	1.0–1.5	PRA, SEAP	(Chou et al., 2007b, 2012a, 2013; Chou and Marousek, 2008)
K355del	pUL97-deficient strain (site-directed mutagenesis) Recombinant virus	304	16	Enzymatic assay, SEAP	(Chou et al., 2013; Prichard et al., 2005)
V356G	Lab-derived strain[a] (under CPV) Recombinant virus	108	5.5	SEAP	Chou et al. (2013)
L397R	Lab-derived strains[a and c] (under 2916W93) Recombinant virus	> 200	1.6	DNA hybridization assay, PRA, SEAP	(Biron et al., 2002; Chou and Marousek, 2008; Chou et al., 2007b; Drew et al., 2006; Evers et al., 2004)
T409M	**Lab-derived strain[b]** **Recombinant virus** **Clinical isolates**	**81**	**0.9**	**PRA, SEAP**	(Chou and Marousek, 2008; Chou et al., 2007b; Houldcroft et al., 2016; Papanicolaou et al., 2018; Strasfeld et al., 2010)
H411L	Lab-derived strain[a] Recombinant virus	69	0.7	SEAP	Chou and Marousek (2008)
H411N	Lab-derived strain[a] Recombinant virus	9	1.0	SEAP	Chou and Marousek (2008)
H411Y	**Lab-derived strain[a]** **Recombinant virus** **Clinical isolates**	**12**	**0.5**	**SEAP**	(Chou and Marousek, 2008; Papanicolaou et al., 2018; Strasfeld et al., 2010)
D456N	Lab-derived strain[c] (under methylenecyclopropane analogue) Recombinant virus	278	12	SEAP	Komazin-Meredith et al. (2014)
V466G	**Clinical isolates (under GCV)** **Recombinant virus**	**321**	**11**	**SEAP**	Chou et al. (2013)
C480R	Lab-derived strain[c] (under methylenecyclopropane analogue) Recombinant virus	243	9	SEAP	Komazin-Meredith et al. (2014)
P521L	**Clinical isolates (under GCV)** **Recombinant virus**	**428–445**	**17**	**SEAP**	Chou et al. (2013)
Y617del	Lab-derived strain[c] (under methylenecyclopropane analogue) Recombinant virus	372	10	SEAP	Komazin-Meredith et al. (2014)
Multiple amino acid changes in pUL97 kinase					
V353A, H411L	Lab-derived strain[a] Recombinant virus	227	1.0	SEAP	Chou and Marousek (2008)
V353A, H411Y	Lab-derived strain[a] Recombinant virus	164	0.7	SEAP	Chou and Marousek (2008)

MBV, maribavir; GCV, ganciclovir; CPV, cyclopropavir; WT, wild-type; PRA, plaque reduction assay; SEAP, secreted alkaline phosphatase; EC_{50}, concentration of antiviral that reduces by 50% the number of plaques or SEAP activity.
Lines in bold show amino acid substitutions identified in clinical specimens.
[a] Exonuclease domain II (D413A) DNA polymerase HCMV mutant.
[b] HCMV clinical strain.
[c] AD169 strain.
Drug-susceptible, EC_{50} ratio < 1.7; Borderline level of resistance, EC_{50} ratio ≥1.7 and ≤ 1.9; Drug-resistant, EC_{50} ratio ≥2.0.

multidrug-resistant phenotype to CPV, GCV and MBV with 18- and 108-fold increase in MBV EC_{50} values, respectively (Chou et al., 2013). Recombinant strains resistant to MBV with amino acid changes at codons 337, 342, 353, 397, 409 and 411 exhibit normal cytopathic effects and viral growth (Chou et al., 2007b, 2012a; Chou and Marousek, 2008). In contrast, the viral replication of V356G mutant is moderately attenuated (Chou et al., 2013). V466G and P521L substitutions identified in clinical specimens isolated from patients treated with GCV (Eckle et al., 2000; Martin et al., 2010a, 2010b) confer high-level resistance to GCV, CPV and MBV (Chou et al., 2013). Furthermore, these two mutants exhibit a pUL97-deficient phenotype with no autophosphorylation and a severe growth defect. D456N and C480R substitutions and Y617del selected in HCMV strain AD169 exposed to methylenecyclopropane analogue confer cross-resistance to GCV and MBV and mutant strains demonstrate abnormal cytopathic effects and severe growth defects (Komazin-Meredith et al., 2014). M460I and M460V substitutions that were already known as conferring resistance to GCV are hypersensitive to MBV with 0.14- and 0.17-fold increases in EC_{50} values (Chou et al., 2007b; Shannon-Lowe and Emery, 2010). The M460I mutant was shown to be less efficient to phosphorylate GCV, to autophosphorylate and to utilize ATP than the wild-type strain (Shannon-Lowe and Emery, 2010). Docking of the MBV molecule in three-dimensional models of the pUL97 kinase structure confirmed that the drug competes with the binding of ATP (Chou and Marousek, 2008). The benzimidazole ring is found between residues 353 and 409–411 that are located between the P-loop and the catalytic loop (Topalis et al., 2018). The majority of amino acid changes conferring resistance to MBV are located at the vicinity of the ATP-binding site of the pUL97 kinase and confer resistance to MBV and not to GCV. Two amino acid changes (L337M and F342S) are located in the ATP-binding loop; F342S substitution confers resistance to both GCV and MBV. A few amino acid substitutions or deletion such as D456N, V466G, C480R, P521L and Y617del are found at distant sites from the ATP-binding loop and confer cross-resistance to GCV and MBV.

The analysis of intra- and interspecies conservation of pUL27 allowed the identification of four conserved regions numbered I to IV

Table 5
Amino acid changes in pUL27 conferring resistance to maribavir.

pUL27	Origin of strain	MBV EC_{50} ratio (mutant/WT)	Assays	References
E22stop	Lab-derived strain[a] Recombinant virus	2.0	Yield reduction assay, SEAP	Chou (2009)
W153R	Lab-derived strain[a] Recombinant virus	1.7	Yield reduction assay, SEAP	Chou (2009)
L193F	Lab-derived strain[a] Recombinant virus	2.6	Yield reduction assay, SEAP	Chou (2009)
C218del	Lab-derived strain[b] Recombinant virus	2.5	Yield reduction assay, SEAP	Chou (2009)
R233S	Lab-derived strains[a and c] (under 2916W93) Recombinant virus	1.8–4.8	Yield reduction assay, SEAP	(Chou et al., 2004, 2012a)
A269T	Lab-derived strain[b] Recombinant virus	2.0	Yield reduction assay, SEAP	Chou (2009)
301-311del	Lab-derived strain[b] Recombinant virus	3.1	Yield reduction assay, SEAP	Chou (2009)
L335P	Lab-derived strain[d]	23	Yield reduction assay	(Komazin et al., 2003a, b)
V353E	Lab-derived strain[a] Recombinant virus	2.1	Yield reduction assay, SEAP	Chou (2009)
W362R	Lab-derived strain[c] (under 2916W93)	1.9	Yield reduction assay	Chou et al. (2004)
W362stop	Lab-derived strain[b] Recombinant virus	2.2	Yield reduction assay, SEAP	Chou (2009)
L426F	Lab-derived strain[a] Recombinant virus	2.2	Yield reduction assay, SEAP	Chou (2009)
Multiple amino acid changes in pUL27				
A406V and C415stop	Lab-derived strain[c] (under 2916W93)	3.5	Yield reduction assay	Chou et al. (2004)
Amino acid changes in pUL27 and pUL97 kinase				
R233S (pUL27) and S337M (pUL97)	Recombinant virus	7.2	SEAP	Chou et al. (2012a)
R233S (pUL27) and S353A (pUL97)	Recombinant virus	27	SEAP	Chou et al. (2012a)

MBV, maribavir; WT, wild-type; SEAP, secreted alkaline phosphatase; EC_{50}, concentration of antiviral that reduces the number of plaques or SEAP activity by 50%.
[a] Exonuclease domain II (D413A) DNA polymerase HCMV mutant.
[b] HCMV clinical strain.
[c] AD169 strain.
[d] Laboratory strain resistant to BDCRB (2-bromo-5,6-dichloro-1-β-D-ribofuranosyl benzimidazole).
Drug-susceptible, EC_{50} ratio < 1.7; Borderline level of resistance, EC_{50} ratio ≥1.7 and ≤ 1.9; Drug-resistant, EC_{50} ratio ≥2.0.

(Hantz et al., 2009). Deletion of *UL27* gene results in a modest half log reduction in viral titers *in vitro* and no apparent effect on viral growth *in vivo* (Prichard et al., 2006). Substitutions in pUL27 were selected after exposure of HCMV strains to MBV and shown to confer low-levels of drug resistance (Fig. 4B; Table 5). These mutations result in single amino acid substitutions and premature stop codons. Frameshift mutations also lead to truncated proteins. The first mutation conferring resistance to MBV detected in the *UL27* gene (resulting in L335P substitution) was identified in a viral isolate resistant to BDCRB and exposed to MBV (Komazin et al., 2003a,b). Other substitutions in pUL27 (R233S, W362R and A406V/C415stop) conferring low-grade resistance to MBV were detected in HCMV AD169 strain exposed to the 2916W93 derivative, a carbocyclic analogue of MBV (Chou et al., 2004). Additional amino acid changes or deletions were also selected in a HCMV clinical isolate (C218del, A269T, 301-311del and W362stop) or in an error-prone exonuclease HCMV mutant (E22stop, W153R, L193F, V353E and L426F) exposed to MBV (Chou, 2009). Combinations of R233S substitution in pUL27 with S337M or S353A substitution in the pUL97 kinase result in approximately 2-fold increase in MBV EC_{50} values over those of the pUL97 single mutants (Chou et al., 2012a). During HCMV replication, pUL27 is predicted to delay cell-cycle progression towards the G_1/S phase. The mechanism involves the promotion of proteasome-dependent degradation of the cellular histone acetyltransferase (HAT), Tip60, which results in an increased expression of the cellular CDK inhibitor, p21 (Reitsma et al., 2011). In contrast, pUL97 which is a viral mimic of CDK, phosphorylates and inactivates Rb to promote cell-cycle progression towards the S phase and to allow viral DNA synthesis. When pUL97 is inhibited by MBV, Rb remains active and S phase genes required for viral DNA synthesis are silent. Thus, the loss of pUL27 could compensate for the loss of pUL97 function explaining the mechanism of MBV resistance (Kamil and Coen, 2011). Furthermore, mutations in *UL27* gene appear spontaneously in genetically pUL97-defective HCMV strains in the absence of drug suggesting a functional compensation for the loss of pUL97 kinase activity (Chou, 2009).

The first clinical isolate harboring mutations conferring resistance to MBV was detected in a heart transplant recipient treated with 400 mg of MBV twice daily (Strasfeld et al., 2010). This patient had a high viral load that was initially reduced by a factor of 50-fold under MBV treatment but rebounded 2 months later. Genotypic testing revealed the emergence of two mutations in *UL97* gene (resulting in T409M and H411Y substitutions) that were already reported to confer resistance to MBV by recombinant phenotyping (Chou and Marousek, 2008). The *UL97* gene was amplified by PCR and cloned in *Escherichia coli*. No clone contained more than one mutation indicating that mutations conferring resistance to MBV emerged from different viral genomes. An immunocompromised pediatric patient with persistent viremia due to viral isolates harboring multiple low frequency mutations in *UL97* and *UL54* genes conferring resistance to GCV, FOS and CDV received salvage therapy with MBV (Houldcroft et al., 2016). A mutation in *UL97* gene resulting in T409M substitution was detected by deep sequencing at a frequency of 2% on day 43 after initiation of therapy and rose to 39% at which time MBV was withdrawn. Furthermore, twenty five of 120 patients enrolled in the phase II study evaluating MBV (400 mg, 800 mg and 1200 mg twice daily) treatment of refractory or drug-resistant HCMV infections in HSCT and SOT recipients developed a recurrent HCMV viremia while on MBV (Papanicolaou et al., 2018). Resistance mutations in *UL97* gene were detected in plasma samples from 13 of these patients (52%); 4, 6 and 3 patients in 400 mg, 800 mg and 1200 mg MBV dose groups, respectively. T409M (in 10 patients) and H411Y (in 3 patients) substitutions that confer resistance to MBV were detected in the pUL97 kinase. Treatment with MBV was continued or resumed for 5 weeks after recurrence. Large clinical trials are needed to evaluate the rate of emergence of MBV resistance as well as to

characterize mutations involved in drug resistance.

5. Conclusions

The search for new inhibitors against HCMV infection has led to the discovery of novel potential targets, the viral terminase complex and the viral pUL97 kinase. The most advanced compounds, LMV and MBV, are safe and well tolerated and have good oral bioavailability. LMV has been recently approved for the prophylaxis of HCMV infection in HSCT recipients. MBV showed efficacy in two phase II clinical studies and is currently evaluated in two phase III trials. Until now, the emergence of viral isolates with mutations conferring resistance to LMV and MBV has been reported in only a few patients. LMV is used off-label as salvage therapy of refractory or drug-resistant HCMV infections. The barrier of resistance to LMV seems to be low compared to other antiviral agents and the level of drug resistance may be very high. Therefore, the emergence of resistance to LMV should be monitored early in patients with a virologic failure. Furthermore, the optimal use of LMV and MBV for the treatment of HCMV infections considering dosing regimens, rate of emergence of drug resistance and characterization of mutations involved in drug resistance should be investigated in large clinical trials. Finally, the benefit of combining LMV and MBV with currently-available antiviral agents or other immunomodulatory compounds such as artesunate and rapamycin should be further evaluated as this strategy may reduce toxicity and the emergence of drug resistance.

Conflicts of interest

The authors declare that there are no competing interests.

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References

Alain, S., Revest, M., Veyer, D., Essig, M., Rerolles, J.P., Rawlinson, W., Mengelle, C., Huynh, A., Kamar, N., Garrigue, I., Kaminski, H., Segard, C., Presne, C., Mazeronw, M.C., Avettant-Fenoel, V., Lecuit, M., Lortholary, O., Coaquette, A., Hantz, S., Leruez-Ville, M., Ploy, M.C., 2013. Maribavir use in practice for cytomegalovirus infection in French transplantation centers. Transplant. Proc. 45, 1603–1607.

Andrei, G., De Clercq, E., Snoeck, R., 2009. Drug targets in cytomegalovirus infection. Infect. Disord. - Drug Targets 9, 201–222.

Avery, R.K., Marty, F.M., Strasfeld, L., Lee, I., Arrieta, A., Chou, S., Tatarowicz, W., Villano, S., 2010. Oral maribavir for treatment of refractory or resistant cytomegalovirus infections in transplant recipients. Transpl. Infect. Dis. 12, 489–496.

Becke, S., Fabre-Mersseman, V., Aue, S., Auerochs, S., Sedmak, T., Wolfrum, U., Strand, D., Marschall, M., Plachter, B., Reyda, S., 2010. Modification of the major tegument protein pp65 of human cytomegalovirus inhibits virus growth and leads to the enhancement of a protein complex with pUL69 and pUL97 in infected cells. J. Gen. Virol. 91, 2531–2541.

Bigley, T.M., Reitsma, J.M., Mirza, S.P., Terhune, S.S., 2013. Human cytomegalovirus pUL97 regulates the viral major immediate early promoter by phosphorylation-mediated disruption of histone deacetylase 1 binding. J. Virol. 87, 7393–7408.

Biron, K.K., Stanat, S.C., Sorrell, J.B., Fyfe, J.A., Keller, P.M., Lambe, C.U., Nelson, D.J., 1985. Metabolic activation of the nucleoside analog 9-[(2-hydroxy-1-(hydroxymethyl)ethoxy]methyl)guanine in human diploid fibroblasts infected with human cytomegalovirus. Proc. Natl. Acad. Sci. U.S.A. 82, 2473–2477.

Biron, K.K., Harvey, R.J., Chamberlain, S.C., Good, S.S., Smith 3rd, A.A., Davis, M.G., Talarico, C.L., Miller, W.H., Ferris, R., Dornsife, R.E., Stanat, S.C., Drach, J.C., Townsend, L.B., Koszalka, G.W., 2002. Potent and selective inhibition of human cytomegalovirus replication by 1263W94, a benzimidazole L-riboside with a unique mode of action. Antimicrob. Agents Chemother. 46, 2365–2372.

Bogner, E., Radsak, K., Stinski, M.F., 1998. The gene product of human cytomegalovirus open reading frame UL56 binds the pac motif and has specific nuclease activity. J. Virol. 72, 2259–2264.

Borst, E.M., Wagner, K., Binz, A., Sodeik, B., Messerle, M., 2008. The essential human cytomegalovirus gene UL52 is required for cleavage-packaging of the viral genome. J. Virol. 82, 2065–2078.

Borst, E.M., Kleine-Albers, J., Gabaev, I., Babic, M., Wagner, K., Binz, A., Degenhardt, I., Kalesse, M., Jonjic, S., Bauerfeind, R., Messerle, M., 2013. The human cytomegalovirus UL51 protein is essential for viral genome cleavage-packaging and interacts with the terminase subunits pUL56 and pUL89. J. Virol. 87, 1720–1732.

Borst, E.M., Bauerfeind, R., Binz, A., Stephan, T.M., Neuber, S., Wagner, K., Steinbruck, L., Sodeik, B., Lenac Rovis, T., Jonjic, S., Messerle, M., 2016. The essential human cytomegalovirus proteins pUL77 and pUL93 are structural components necessary for viral genome encapsidation. J. Virol. 90, 5860–5875.

Britt, W.J., Prichard, M.N., 2018. New therapies for human cytomegalovirus infections. Antivir. Res. 159, 153–174.

Campos, A.B., Ribeiro, J., Boutolleau, D., Sousa, H., 2016. Human cytomegalovirus antiviral drug resistance in hematopoietic stem cell transplantation: current state of the art. Rev. Med. Virol. 26, 161–182.

Champier, G., Hantz, S., Couvreux, A., Stuppfler, S., Mazeron, M.C., Bouaziz, S., Denis, F., Alain, S., 2007. New functional domains of human cytomegalovirus pUL89 predicted by sequence analysis and three-dimensional modelling of the catalytic site DEXDc. Antivir. Ther. 12, 217–232.

Champier, G., Couvreux, A., Hantz, S., Rametti, A., Mazeron, M.C., Bouaziz, S., Denis, F., Alain, S., 2008. Putative functional domains of human cytomegalovirus pUL56 involved in dimerization and benzimidazole D-ribonucleoside activity. Antivir. Ther. 13, 643–654.

Chan, J.H., Chamberlain, S.D., Biron, K.K., Davis, M.G., Harvey, R.J., Selleseth, D.W., Dornsife, R.E., Dark, E.H., Frick, L.W., Townsend, L.B., Drach, J.C., Koszalka, G.W., 2000. Synthesis and evaluation of a series of 2'-deoxy analogues of the antiviral agent 5,6-dichloro-2-isopropylamino-1-(beta-L-ribofuranosyl)-1H-benzimidazole (1263W94). Nucleos Nucleot. Nucleic Acids 19, 101–123.

Chemaly, R.F., Ullmann, A.J., Stoelben, S., Richard, M.P., Bornhauser, M., Groth, C., Einsele, H., Silverman, M., Mullane, K.M., Brown, J., Nowak, H., Kolling, K., Stobernack, H.P., Lischka, P., Zimmermann, H., Rubsamen-Schaeff, H., Champlin, R.E., Ehninger, G., Team, A.S., 2014. Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. N. Engl. J. Med. 370, 1781–1789.

Cherrier, L., Nasar, A., Goodlet, K.J., Nailor, M.D., Tokman, S., Chou, S., 2018. Emergence of letermovir resistance in a lung transplant recipient with ganciclovir-resistant cytomegalovirus infection. Am. J. Transplant. 18, 3060–3064.

Chong, P.P., Teiber, D., Prokesch, B.C., Arasaratnam, R.J., Peltz, M., Drazner, M.H., Garg, S., 2018. Letermovir successfully used for secondary prophylaxis in a heart transplant recipient with ganciclovir-resistant cytomegalovirus syndrome (UL97 mutation). Transpl. Infect. Dis. 20, e12965.

Chou, S., 2009. Diverse cytomegalovirus UL27 mutations adapt to loss of viral UL97 kinase activity under maribavir. Antimicrob. Agents Chemother. 53, 81–85.

Chou, S., 2015. Rapid in vitro evolution of human cytomegalovirus UL56 mutations that confer letermovir resistance. Antimicrob. Agents Chemother. 59, 6588–6593.

Chou, S., 2017a. Comparison of cytomegalovirus terminase gene mutations selected after exposure to three distinct inhibitor compounds. Antimicrob. Agents Chemother. 61 e01325-17.

Chou, S., 2017b. A third component of the human cytomegalovirus terminase complex is involved in letermovir resistance. Antivir. Res. 148, 1–4.

Chou, S., Marousek, G.I., 2006. Maribavir antagonizes the antiviral action of ganciclovir on human cytomegalovirus. Antimicrob. Agents Chemother. 50, 3470–3472.

Chou, S., Marousek, G.I., 2008. Accelerated evolution of maribavir resistance in a cytomegalovirus exonuclease domain II mutant. J. Virol. 82, 246–253.

Chou, S., Marousek, G.I., Senters, A.E., Davis, M.G., Biron, K.K., 2004. Mutations in the human cytomegalovirus UL27 gene that confer resistance to maribavir. J. Virol. 78, 7124–7130.

Chou, S., Van Wechel, L.C., Lichy, H.M., Marousek, G.I., 2005. Phenotyping of cytomegalovirus drug resistance mutations by using recombinant viruses incorporating a reporter gene. Antimicrob. Agents Chemother. 49, 2710–2715.

Chou, S., Marousek, G.I., Van Wechel, L.C., Li, S., Weinberg, A., 2007a. Growth and drug resistance phenotypes resulting from cytomegalovirus DNA polymerase region III mutations observed in clinical specimens. Antimicrob. Agents Chemother. 51, 4160–4162.

Chou, S., Wechel, L.C., Marousek, G.I., 2007b. Cytomegalovirus UL97 kinase mutations that confer maribavir resistance. J. Infect. Dis. 196, 91–94.

Chou, S., Hakki, M., Villano, S., 2012a. Effects on maribavir susceptibility of cytomegalovirus UL97 kinase ATP binding region mutations detected after drug exposure in vitro and in vivo. Antivir. Res. 95, 88–92.

Chou, S., Marousek, G., Bowlin, T.L., 2012b. Cyclopropavir susceptibility of cytomegalovirus DNA polymerase mutants selected after antiviral drug exposure. Antimicrob. Agents Chemother. 56, 197–201.

Chou, S., Ercolani, R.J., Marousek, G., Bowlin, T.L., 2013. Cytomegalovirus UL97 kinase catalytic domain mutations that confer multidrug resistance. Antimicrob. Agents Chemother. 57, 3375–3379.

Chou, S., Ercolani, R.J., Derakhchan, K., 2018a. Antiviral activity of maribavir in combination with other drugs active against human cytomegalovirus. Antivir. Res. 157, 128–133.

Chou, S., Satterwhite, L.E., Ercolani, R.J., 2018b. A new locus of drug resistance in the human cytomegalovirus UL56 gene revealed by in vitro exposure to letermovir and ganciclovir. Antimicrob. Agents Chemother. 62 e00922-18.

Chrisp, P., Clissold, S.P., 1991. Foscarnet. A review of its antiviral activity, pharmacokinetic properties and therapeutic use in immunocompromised patients with cytomegalovirus retinitis. Drugs 41, 104–129.

Cihlar, T., Chen, M.S., 1996. Identification of enzymes catalyzing two-step phosphorylation of cidofovir and the effect of cytomegalovirus infection on their activities in host cells. Mol. Pharmacol. 50, 1502–1510.

Dittmer, A., Drach, J.C., Townsend, L.B., Fischer, A., Bogner, E., 2005. Interaction of the putative human cytomegalovirus portal protein pUL104 with the large terminase subunit pUL56 and its inhibition by benzimidazole-D-ribonucleosides. J. Virol. 79, 14660–14667.

Drew, W.L., Miner, R.C., Marousek, G.I., Chou, S., 2006. Maribavir sensitivity of cytomegalovirus isolates resistant to ganciclovir, cidofovir or foscarnet. J. Clin. Virol. 37, 124–127.
Drouot, E., Piret, J., Boivin, G., 2013. Novel method based on "en passant" mutagenesis coupled with a Gaussia luciferase reporter assay for studying the combined effects of human cytomegalovirus mutations. J. Clin. Microbiol. 51, 3216–3224.
Drouot, E., Piret, J., Lebel, M.H., Boivin, G., 2014. Characterization of multiple cytomegalovirus drug resistance mutations detected in a hematopoietic stem cell transplant recipient by recombinant phenotyping. J. Clin. Microbiol. 52, 4043–4046.
Drouot, E., Piret, J., Boivin, G., 2016. Artesunate demonstrates in vitro synergism with several antiviral agents against human cytomegalovirus. Antivir. Ther. 21, 535–539.
Eckle, T., Prix, L., Jahn, G., Klingebiel, T., Handgretinger, R., Selle, B., Hamprecht, K., 2000. Drug-resistant human cytomegalovirus infection in children after allogeneic stem cell transplantation may have different clinical outcomes. Blood 96, 3286–3289.
Erb-Zohar, K., Kropeit, D., Scheuenpflug, J., Stobernack, H.P., Hulskotte, E., van Schanke, A., Zimmermann, H., Rubsamen-Schaeff, H., 2017. Intravenous hydroxypropyl beta-cyclodextrin formulation of letermovir: a phase I, randomized, single-ascending, and multiple-dose trial. Clin. Transl. Sci. 10, 487–495.
Evers, D.L., Komazin, G., Ptak, R.G., Shin, D., Emmer, B.T., Townsend, L.B., Drach, J.C., 2004. Inhibition of human cytomegalovirus replication by benzimidazole nucleosides involves three distinct mechanisms. Antimicrob. Agents Chemother. 48, 3918–3927.
Gagelmann, N., Ljungman, P., Styczynski, J., Kroger, N., 2018. Comparative efficacy and safety of different antiviral agents for cytomegalovirus prophylaxis in allogeneic hematopoietic cell transplantation: a systematic review and meta-analysis. Biol. Blood Marrow Transplant. 24, 2101–2109.
Gentry, B.G., Bogner, E., Drach, J.C., 2019. Targeting the terminase: an important step forward in the treatment and prophylaxis of human cytomegalovirus infections. Antivir. Res. 161, 116–124.
Goldner, T., Hewlett, G., Ettischer, N., Ruebsamen-Schaeff, H., Zimmermann, H., Lischka, P., 2011. The novel anticytomegalovirus compound AIC246 (Letermovir) inhibits human cytomegalovirus replication through a specific antiviral mechanism that involves the viral terminase. J. Virol. 85, 10884–10893.
Goldner, T., Hempel, C., Ruebsamen-Schaeff, H., Zimmermann, H., Lischka, P., 2014. Geno- and phenotypic characterization of human cytomegalovirus mutants selected in vitro after letermovir (AIC246) exposure. Antimicrob. Agents Chemother. 58, 610–613.
Goldwater, D.R., Dougherty, C., Schumacher, M., Villano, S.A., 2008. Effect of ketoconazole on the pharmacokinetics of maribavir in healthy adults. Antimicrob. Agents Chemother. 52, 1794–1798.
Hamirally, S., Kamil, J.P., Ndassa-Colday, Y.M., Lin, A.J., Jahng, W.J., Baek, M.C., Noton, S., Silva, L.A., Simpson-Holley, M., Knipe, D.M., Golan, D.E., Marto, J.A., Coen, D.M., 2009. Viral mimicry of Cdc2/cyclin-dependent kinase 1 mediates disruption of nuclear lamina during human cytomegalovirus nuclear egress. PLoS Pathog. 5, e1000275.
Hanks, S.K., Quinn, A.M., Hunter, T., 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241, 42–52.
Hantz, S., Couvreux, A., Champier, G., Trapes, L., Cotin, S., Denis, F., Bouaziz, S., Alain, S., 2009. Conserved domains and structure prediction of human cytomegalovirus UL27 protein. Antivir. Ther. 14, 663–672.
He, Z., He, Y.S., Kim, Y., Chu, L., Ohmstede, C., Biron, K.K., Coen, D.M., 1997. The human cytomegalovirus UL97 protein is a protein kinase that autophosphorylates on serines and threonines. J. Virol. 71, 405–411.
Hostetler, K.Y., 2010. Synthesis and early development of hexadecyloxypropylcidofovir: an oral antipoxvirus nucleoside phosphonate. Viruses 2, 2213–2225.
Houldcroft, C.J., Bryant, J.M., Depledge, D.P., Margetts, B.K., Simmonds, J., Nicolaou, S., Tutill, H.J., Williams, R., Worth, A.J., Marks, S.D., Veys, P., Whittaker, E., Breuer, J., 2016. Detection of low frequency multi-drug resistance and novel putative maribavir resistance in immunocompromised pediatric patients with cytomegalovirus. Front. Microbiol. 7, 1317.
Hwang, J.S., Bogner, E., 2002. ATPase activity of the terminase subunit pUL56 of human cytomegalovirus. J. Biol. Chem. 277, 6943–6948.
James, S.H., Hartline, C.B., Harden, E.A., Driebe, E.M., Schupp, J.M., Engelthaler, D.M., Keim, P.S., Bowlin, T.L., Kern, E.R., Prichard, M.N., 2011. Cyclopropavir inhibits the normal function of the human cytomegalovirus UL97 kinase. Antimicrob. Agents Chemother. 55, 4682–4691.
Kamil, J.P., Coen, D.M., 2007. Human cytomegalovirus protein kinase UL97 forms a complex with the tegument phosphoprotein pp65. J. Virol. 81, 10659–10668.
Kamil, J.P., Coen, D.M., 2011. HATs on for drug resistance. Cell Host Microbe 9, 85–87.
Kaul, D.R., Stoelben, S., Cober, E., Ojo, T., Sandusky, E., Lischka, P., Zimmermann, H., Rubsamen-Schaeff, H., 2011. First report of successful treatment of multidrug-resistant cytomegalovirus disease with the novel anti-CMV compound AIC246. Am. J. Transplant. 11, 1079–1084.
Knoll, B.M., Seiter, K., Phillips, A., Soave, R., 2018. Breakthrough cytomegalovirus pneumonia in hematopoietic stem cell transplant recipient on letermovir prophylaxis. Bone Marrow Transplant.
Komazin, G., Ptak, R.G., Emmer, B.T., Townsend, L.B., Drach, J.C., 2003a. Resistance of human cytomegalovirus to D- and L-ribosyl benzimidazoles as a tool to identify potential targets for antiviral drugs. Nucleos Nucleot. Nucleic Acids 22, 1725–1727.
Komazin, G., Ptak, R.G., Emmer, B.T., Townsend, L.B., Drach, J.C., 2003b. Resistance of human cytomegalovirus to the benzimidazole L-ribonucleoside maribavir maps to UL27. J. Virol. 77, 11499–11506.
Komazin-Meredith, G., Chou, S., Prichard, M.N., Hartline, C.B., Cardinale, S.C., Comeau, K., Williams, J.D., Khan, A.R., Peet, N.P., Bowlin, T.L., 2014. Human cytomegalovirus UL97 kinase is involved in the mechanism of action of methylenecyclopropane analogs with 6-ether and -thioether substitutions. Antimicrob. Agents Chemother. 58, 274–278.
Koppen-Rung, P., Dittmer, A., Bogner, E., 2016. Intracellular distribution of capsid-associated pUL77 of human cytomegalovirus and interactions with packaging proteins and pUL93. J. Virol. 90, 5876–5885.
Koszalka, G.W., Johnson, N.W., Good, S.S., Boyd, L., Chamberlain, S.C., Townsend, L.B., Drach, J.C., Biron, K.K., 2002. Preclinical and toxicology studies of 1263W94, a potent and selective inhibitor of human cytomegalovirus replication. Antimicrob. Agents Chemother. 46, 2373–2380.
Kotton, C.N., Kumar, D., Caliendo, A.M., Huprikar, S., Chou, S., Danziger-Isakov, L., Humar, A., The Transplantation Society International CMV Consensus Group, 2018. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation 102, 900–931.
Kropeit, D., McCormick, D., Erb-Zohar, K., Moiseev, V.S., Kobalava, Z.D., Stobernack, H.P., Zimmermann, H., Rubsamen-Schaeff, H., 2017a. Pharmacokinetics and safety of the anti-human cytomegalovirus drug letermovir in subjects with hepatic impairment. Br. J. Clin. Pharmacol. 83, 2678–2686.
Kropeit, D., Scheuenpflug, J., Erb-Zohar, K., Halabi, A., Stobernack, H.P., Hulskotte, E.G.J., van Schanke, A., Zimmermann, H., Rubsamen-Schaeff, H., 2017b. Pharmacokinetics and safety of letermovir, a novel anti-human cytomegalovirus drug, in patients with renal impairment. Br. J. Clin. Pharmacol. 83, 1944–1953.
Kropeit, D., von Richter, O., Stobernack, H.P., Rubsamen-Schaeff, H., Zimmermann, H., 2018. Pharmacokinetics and safety of letermovir coadministered with cyclosporine A or tacrolimus in healthy subjects. Clin. Pharmacol. Drug Dev. 7, 9–21.
Krosky, P.M., Baek, M.C., Coen, D.M., 2003a. The human cytomegalovirus UL97 protein kinase, an antiviral drug target, is required at the stage of nuclear egress. J. Virol. 77, 905–914.
Krosky, P.M., Baek, M.C., Jahng, W.J., Barrera, I., Harvey, R.J., Biron, K.K., Coen, D.M., Sethna, P.B., 2003b. The human cytomegalovirus UL44 protein is a substrate for the UL97 protein kinase. J. Virol. 77, 7720–7727.
Lalezari, J.P., Aberg, J.A., Wang, L.H., Wire, M.B., Miner, R., Snowden, W., Talarico, C.L., Shaw, S., Jacobson, M.A., Drew, W.L., 2002. Phase I dose escalation trial evaluating the pharmacokinetics, anti-human cytomegalovirus (HCMV) activity, and safety of 1263W94 in human immunodeficiency virus-infected men with asymptomatic HCMV shedding. Antimicrob. Agents Chemother. 46, 2969–2976.
Ligat, G., Cazal, R., Hantz, S., Alain, S., 2018. The human cytomegalovirus terminase complex as an antiviral target: a close-up view. FEMS Microbiol. Rev. 42, 137–145.
Lischka, P., Hewlett, G., Wunberg, T., Baumeister, J., Paulsen, D., Goldner, T., Ruebsamen-Schaeff, H., Zimmermann, H., 2010. In vitro and in vivo activities of the novel anticytomegalovirus compound AIC246. Antimicrob. Agents Chemother. 54, 1290–1297.
Lischka, P., Michel, D., Zimmermann, H., 2016. Characterization of cytomegalovirus breakthrough events in a phase 2 prophylaxis trial of letermovir (AIC246, MK 8228). J. Infect. Dis. 213, 23–30.
Lurain, N.S., Chou, S., 2010. Antiviral drug resistance of human cytomegalovirus. Clin. Microbiol. Rev. 23, 689–712.
Ma, J.D., Nafziger, A.N., Villano, S.A., Gaedigk, A., Bertino Jr., J.S., 2006. Maribavir pharmacokinetics and the effects of multiple-dose maribavir on cytochrome P450 (CYP) 1A2, CYP 2C9, CYP 2C19, CYP 2D6, CYP 3A, N-acetyltransferase-2, and xanthine oxidase activities in healthy adults. Antimicrob. Agents Chemother. 50, 1130–1135.
Maertens, J.A., Cordonnier, C., Jaksch, P., Poiré, X., Wu, J.J., Wijatyk, A., Saliba, F., Witzke, O., Villano, S., 2016. Maribavir versus valganciclovir for preemptive treatment of cytomegalovirus (CMV) viremia: a randomized, dose-ranging, phase 2 study among hematopoietic stem cell transplant (SCT) and solid organ transplant (SOT) recipients. Open Forum Infect. Dis. 3 (Suppl. 1), 2287.
Marfori, J.E., Exner, M.M., Marousek, G.I., Chou, S., Drew, W.L., 2007. Development of new cytomegalovirus UL97 and DNA polymerase mutations conferring drug resistance after valganciclovir therapy in allogeneic stem cell recipients. J. Clin. Virol. 38, 120–125.
Marschall, M., Stein-Gerlach, M., Freitag, M., Kupfer, R., van Den Bogaard, M., Stamminger, T., 2001. Inhibitors of human cytomegalovirus replication drastically reduce the activity of the viral protein kinase pUL97. J. Gen. Virol. 82, 1439–1450.
Marschall, M., Freitag, M., Suchy, P., Romaker, D., Kupfer, R., Hanke, M., Stamminger, T., 2003. The protein kinase pUL97 of human cytomegalovirus interacts with and phosphorylates the DNA polymerase processivity factor pUL44. Virology 311, 60–71.
Marschall, M., Stamminger, T., Urban, A., Wildum, S., Ruebsamen-Schaeff, H., Zimmermann, H., Lischka, P., 2012. In vitro evaluation of the activities of the novel anticytomegalovirus compound AIC246 (letermovir) against herpesviruses and other human pathogenic viruses. Antimicrob. Agents Chemother. 56, 1135–1137.
Marshall, W.L., McCrea, J.B., Macha, S., Menzel, K., Liu, F., van Schanke, A., de Haes, J.I.U., Hussaini, A., Jordan, H.R., Drexel, M., Kantesaria, B.S., Tsai, C., Cho, C.R., Hulskotte, E.G.J., Butterton, J.R., Iwamoto, M., 2018. Pharmacokinetics and tolerability of letermovir coadministered with azole antifungals (posaconazole or voriconazole) in healthy subjects. J. Clin. Pharmacol. 58, 897–904.
Martin, M., Goyette, N., Boivin, G., 2010a. Contrasting effects on ganciclovir susceptibility and replicative capacity of two mutations at codon 466 of the human cytomegalovirus UL97 gene. J. Clin. Virol. 49, 296–298.
Martin, M., Goyette, N., Ives, J., Boivin, G., 2010b. Incidence and characterization of cytomegalovirus resistance mutations among pediatric solid organ transplant patients who received valganciclovir prophylaxis. J. Clin. Virol. 47, 321–324.
Marty, F.M., Boeckh, M., 2011. Maribavir and human cytomegalovirus-what happened in the clinical trials and why might the drug have failed? Curr. Opin. Virol. 1, 555–562.
Marty, F.M., Ljungman, P., Papanicolaou, G.A., Winston, D.J., Chemaly, R.F., Strasfeld, L., Young, J.A., Rodriguez, T., Maertens, J., Schmitt, M., Einsele, H., Ferrant, A., Lipton, J.H., Villano, S.A., Chen, H., Boeckh, M., Maribavir -300 Clinical Study Group, 2011. Maribavir prophylaxis for prevention of cytomegalovirus disease in recipients of allogeneic stem-cell transplants: a phase 3, double-blind, placebo-controlled,

randomised trial. Lancet Infect. Dis. 11, 284–292.
Marty, F.M., Winston, D.J., Rowley, S.D., Vance, E., Papanicolaou, G.A., Mullane, K.M., Brundage, T.M., Robertson, A.T., Godkin, S., Mommeja-Marin, H., Boeckh, M., CMX001-201 Clinical Study Group, 2013. CMX001 to prevent cytomegalovirus disease in hematopoietic-cell transplantation. N. Engl. J. Med. 369, 1227–1236.
Marty, F.M., Ljungman, P., Chemaly, R.F., Maertens, J., Dadwal, S.S., Duarte, R.F., Haider, S., Ullmann, A.J., Katayama, Y., Brown, J., Mullane, K.M., Boeckh, M., Blumberg, E.A., Einsele, H., Snydman, D.R., Kanda, Y., DiNubile, M.J., Teal, V.L., Wan, H., Murata, Y., Kartsonis, N.A., Leavitt, R.Y., Badshah, C., 2017. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N. Engl. J. Med. 377, 2433–2444.
Marty, F.M., Winston, D.J., Chemaly, R.F., Mullane, K.M., Shore, T.B., Papanicolaou, G.A., Chittick, G., Brundage, T.M., Wilson, C., Morrison, M.E., Foster, S.A., Nichols, W.G., Boeckh, M.J., SUPPRESS Trial Clinical Study Group, 2019. A randomized, double-blind, placebo-controlled phase 3 trial of oral brincidofovir for cytomegalovirus prophylaxis in allogeneic hematopoietic-cell transplantation. Biol. Blood Marrow Transplant. 25, 369–381.
McSharry, J.J., McDonough, A., Olson, B., Hallenberger, S., Reefschlaeger, J., Bender, W., Drusano, G.L., 2001. Susceptibilities of human cytomegalovirus clinical isolates to BAY38-4766, BAY43-9695, and ganciclovir. Antimicrob. Agents Chemother. 45, 2925–2927.
Merck Sharp & Dohme Corp, 2017. PREVYMIS: Package Insert and Label Information. https://druginserts.com/lib/rx/meds/prevymis-1/, Accessed date: 16 January 2019.
Michel, D., Pavic, I., Zimmermann, A., Haupt, E., Wunderlich, K., Heuschmid, M., Mertens, T., 1996. The UL97 gene product of human cytomegalovirus is an early-late protein with a nuclear localization but is not a nucleoside kinase. J. Virol. 70, 6340–6346.
Mitchell, M.S., Matsuzaki, S., Imai, S., Rao, V.B., 2002. Sequence analysis of bacteriophage T4 DNA packaging/terminase genes 16 and 17 reveals a common ATPase center in the large subunit of viral terminases. Nucleic Acids Res. 30, 4009–4021.
Nagelschmitz, J., Moeller, J.G., Stass, H., Wandel, C., Kuhlmann, J., 1999. Safety, tolerability and pharmacokinetics of single oral doses of BAY 38-4766–a novel, non-nucleosidic inhibitor of human cytomegalovirus replication–in healthy male subjects. In: 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco.
Neuber, S., Wagner, K., Goldner, T., Lischka, P., Steinbrueck, L., Messerle, M., Borst, E.M., 2017. Mutual interplay between the human cytomegalovirus terminase subunits pUL51, pUL56, and pUL89 promotes terminase complex formation. J. Virol. 91 e02384-16.
O'Brien, M.S., Markovich, K.C., Selleseth, D., DeVita, A.V., Sethna, P., Gentry, B.G., 2018. In vitro evaluation of current and novel antivirals in combination against human cytomegalovirus. Antivir. Res. 158, 255–263.
Papanicolaou, G.A., Silveira, F.P., Langston, A.A., Pereira, M.R., Avery, R.K., Uknis, M., Wijatyk, A., Wu, J., Boeckh, M., Marty, F.M., Villano, S., 2018. Maribavir for refractory or resistant cytomegalovirus infections in hematopoietic-cell or solid-organ transplant recipients: a randomized, dose-ranging, double-blind, phase 2 study. Clin. Infect. Dis.
Pescovitz, M.D., Rabkin, J., Merion, R.M., Paya, C.V., Pirsch, J., Freeman, R.B., O'Grady, J., Robinson, C., To, Z., Wren, K., Banken, L., Buhles, W., Brown, F., 2000. Valganciclovir results in improved oral absorption of ganciclovir in liver transplant recipients. Antimicrob. Agents Chemother. 44, 2811–2815.
Pescovitz, M.D., Bloom, R., Pirsch, J., Johnson, J., Gelone, S., Villano, S.A., 2009. A randomized, double-blind, pharmacokinetic study of oral maribavir with tacrolimus in stable renal transplant recipients. Am. J. Transplant. 9, 2324–2330.
Pilorge, L., Burrel, S., Ait-Arkoub, Z., Agut, H., Boutolleau, D., 2014. Human cytomegalovirus (CMV) susceptibility to currently approved antiviral drugs does not impact on CMV terminase complex polymorphism. Antivir. Res. 111, 8–12.
Piret, J., Goyette, N., Boivin, G., 2017. Drug susceptibility and replicative capacity of multidrug-resistant recombinant human cytomegalovirus harboring mutations in UL56 and UL54 genes. Antimicrob. Agents Chemother. 61 e01044-17.
Prichard, M.N., 2009. Function of human cytomegalovirus UL97 kinase in viral infection and its inhibition by maribavir. Rev. Med. Virol. 19, 215–229.
Prichard, M.N., Gao, N., Jairath, S., Mulamba, G., Krosky, P., Coen, D.M., Parker, B.O., Pari, G.S., 1999. A recombinant human cytomegalovirus with a large deletion in UL97 has a severe replication deficiency. J. Virol. 73, 5663–5670.
Prichard, M.N., Britt, W.J., Daily, S.L., Hartline, C.B., Kern, E.R., 2005. Human cytomegalovirus UL97 Kinase is required for the normal intranuclear distribution of pp65 and virion morphogenesis. J. Virol. 79, 15494–15502.
Prichard, M.N., Quenelle, D.C., Bidanset, D.J., Komazin, G., Chou, S., Drach, J.C., Kern, E.R., 2006. Human cytomegalovirus UL27 is not required for viral replication in human tissue implanted in SCID mice. Virol. J. 3, 18.
Reefschlaeger, J., Bender, W., Hallenberger, S., Weber, O., Eckenberg, P., Goldmann, S., Haerter, M., Buerger, I., Trappe, J., Herrington, J.A., Haebich, D., Ruebsamen-Waigmann, H., 2001. Novel non-nucleoside inhibitors of cytomegaloviruses (BAY 38-4766): in vitro and in vivo antiviral activity and mechanism of action. J. Antimicrob. Chemother. 48, 757–767.
Reim, N.I., Kamil, J.P., Wang, D., Lin, A., Sharma, M., Ericsson, M., Pesola, J.M., Golan, D.E., Coen, D.M., 2013. Inactivation of retinoblastoma protein does not overcome the requirement for human cytomegalovirus UL97 in lamina disruption and nuclear egress. J. Virol. 87, 5019–5027.
Reitsma, J.M., Savaryn, J.P., Faust, K., Sato, H., Halligan, B.D., Terhune, S.S., 2011. Antiviral inhibition targeting the HCMV kinase pUL97 requires pUL27-dependent degradation of Tip60 acetyltransferase and cell-cycle arrest. Cell Host Microbe 9, 103–114.
Scheffczik, H., Savva, C.G., Holzenburg, A., Kolesnikova, L., Bogner, E., 2002. The terminase subunits pUL56 and pUL89 of human cytomegalovirus are DNA-metabolizing proteins with toroidal structure. Nucleic Acids Res. 30, 1695–1703.
Scholz, B., Rechter, S., Drach, J.C., Townsend, L.B., Bogner, E., 2003. Identification of the ATP-binding site in the terminase subunit pUL56 of human cytomegalovirus. Nucleic Acids Res. 31, 1426–1433.
Shannon-Lowe, C.D., Emery, V.C., 2010. The effects of maribavir on the autophosphorylation of ganciclovir resistant mutants of the cytomegalovirus UL97 protein. Herpesviridae 1, 4.
Sharma, M., Bender, B.J., Kamil, J.P., Lye, M.F., Pesola, J.M., Reim, N.I., Hogle, J.M., Coen, D.M., 2015. Human cytomegalovirus UL97 phosphorylates the viral nuclear egress complex. J. Virol. 89, 523–534.
Stoelben, S., Arns, W., Renders, L., Hummel, J., Muhlfeld, A., Stangl, M., Fischereder, M., Gwinner, W., Suwelack, B., Witzke, O., Durr, M., Beelen, D.W., Michel, D., Lischka, P., Zimmermann, H., Rubsamen-Schaeff, H., Budde, K., 2014. Preemptive treatment of Cytomegalovirus infection in kidney transplant recipients with letermovir: results of a Phase 2a study. Transpl. Int. 27, 77–86.
Strasfeld, L., Lee, I., Tatarowicz, W., Villano, S., Chou, S., 2010. Virologic characterization of multidrug-resistant cytomegalovirus infection in 2 transplant recipients treated with maribavir. J. Infect. Dis. 202, 104–108.
Swan, S.K., Smith, W.B., Marbury, T.C., Schumacher, M., Dougherty, C., Mico, B.A., Villano, S.A., 2007. Pharmacokinetics of maribavir, a novel oral anticytomegalovirus agent, in subjects with varying degrees of renal impairment. J. Clin. Pharmacol. 47, 209–217.
Thomas, M., Rechter, S., Milbradt, J., Auerochs, S., Muller, R., Stamminger, T., Marschall, M., 2009. Cytomegaloviral protein kinase pUL97 interacts with the nuclear mRNA export factor pUL69 to modulate its intranuclear localization and activity. J. Gen. Virol. 90, 567–578.
Topalis, D., Gillemot, S., Snoeck, R., Andrei, G., 2018. Thymidine kinase and protein kinase in drug-resistant herpesviruses: heads of a lernaean hydra. Drug Resist. Updates 37, 1–16.
Turner, N., Strand, A., Grewal, D.S., Cox, G., Arif, S., Baker, A.W., Maziarz, E.K., Saullo, J.H., Wolfe, C.R., 2019. Use of letermovir as salvage therapy for drug-resistant CMV retinitis: a case series. Antimicrob. Agents Chemother e02337-18.
Underwood, M.R., Harvey, R.J., Stanat, S.C., Hemphill, M.L., Miller, T., Drach, J.C., Townsend, L.B., Biron, K.K., 1998. Inhibition of human cytomegalovirus DNA maturation by a benzimidazole ribonucleoside is mediated through the UL89 gene product. J. Virol. 72, 717–725.
Underwood, M.R., Ferris, R.G., Selleseth, D.W., Davis, M.G., Drach, J.C., Townsend, L.B., Biron, K.K., Boyd, F.L., 2004. Mechanism of action of the ribopyranoside benzimidazole GW275175X against human cytomegalovirus. Antimicrob. Agents Chemother. 48, 1647–1651.
Wang, L.H., Peck, R.W., Yin, Y., Allanson, J., Wiggs, R., Wire, M.B., 2003. Phase I safety and pharmacokinetic trials of 1263W94, a novel oral anti-human cytomegalovirus agent, in healthy and human immunodeficiency virus-infected subjects. Antimicrob. Agents Chemother. 47, 1334–1342.
Wildum, S., Zimmermann, H., Lischka, P., 2015. In vitro drug combination studies of Letermovir (AIC246, MK-8228) with approved anti-human cytomegalovirus (HCMV) and anti-HIV compounds in inhibition of HCMV and HIV replication. Antimicrob. Agents Chemother. 59, 3140–3148.
Williams, S.L., Hartline, C.B., Kushner, N.L., Harden, E.A., Bidanset, D.J., Drach, J.C., Townsend, L.B., Underwood, M.R., Biron, K.K., Kern, E.R., 2003. In vitro activities of benzimidazole D- and L-ribonucleosides against herpesviruses. Antimicrob. Agents Chemother. 47, 2186–2192.
Winston, D.J., Young, J.A., Pullarkat, V., Papanicolaou, G.A., Vij, R., Vance, E., Alangaden, G.J., Chemaly, R.F., Petersen, F., Chao, N., Klein, J., Sprague, K., Villano, S.A., Boeckh, M., 2008. Maribavir prophylaxis for prevention of cytomegalovirus infection in allogeneic stem cell transplant recipients: a multicenter, randomized, double-blind, placebo-controlled, dose-ranging study. Blood 111, 5403–5410.
Winston, D.J., Saliba, F., Blumberg, E., Abouljoud, M., Garcia-Diaz, J.B., Goss, J.A., Clough, L., Avery, R., Limaye, A.P., Ericzon, B.G., Navasa, M., Troisi, R.I., Chen, H., Villano, S.A., Uknis, M.E., 1263-301 Clinical Study Group, 2012. Efficacy and safety of maribavir dosed at 100 mg orally twice daily for the prevention of cytomegalovirus disease in liver transplant recipients: a randomized, double-blind, multicenter controlled trial. Am. J. Transplant. 12, 3021–3030.
Xiong, X., Smith, J.L., Kim, C., Huang, E.S., Chen, M.S., 1996. Kinetic analysis of the interaction of cidofovir diphosphate with human cytomegalovirus DNA polymerase. Biochem. Pharmacol. 51, 1563–1567.
Yeung, C.K., Shen, D.D., Thummel, K.E., Himmelfarb, J., 2014. Effects of chronic kidney disease and uremia on hepatic drug metabolism and transport. Kidney Int. 85, 522–528.
Zarrouk, K., Piret, J., Boivin, G., 2017. Herpesvirus DNA polymerases: structures, functions and inhibitors. Virus Res. 234, 177–192.