Goldner *et al.*, 2011; Marschall *et al.*, 2011). Letermovir demonstrated approximately 400-fold greater potency than ganciclovir against two HCMV strains in both cell cytopathic effect reduction assays (CPE-RAs) and green fluorescence reduction assays (GFP-RAs), with EC_{50} values ranging from 3.8 to 5.0 nM (Goldner *et al.*, 2011; Lischka *et al.,* 2010). The relatively stable EC_{90} values (range: 5.0–7.1 nM) in comparison to the EC_{50} values in the GFP-RA assays are evidence of a steep dose–response curve (Lischka *et al.,* 2010). In contrast to maribavir, the potency of letermovir does not appear to be significantly affected by the human fibroblast line used in antiviral assays (Chou *et al.* 2006; Lischka *et al.,* 2010).

In traditional plaque reduction assays against 17 clinical HCMV isolates, the letermovir EC_{50} ranged from 0.9 to 3.1 nM, and letermovir was approximately 1000-fold more potent than ganciclovir against the same strains (Marschall *et al.*, 2011). After removal of letermovir from the cell culture medium, HCMV viral replication rapidly resumes and reaches levels similar to that of untreated controls within less than 48 hours. In comparison, viral levels in ganciclovir-treated cells did not return to those seen in untreated cells until approximately 100 hours after removal, suggesting that the postantiviral effect of letermovir is probably less prolonged than other antiviral agents (Lischka *et al.,* 2010). Letermovir also demonstrates an ability to reduce the spread of virus in co-cultivated fibroblasts, reducing the number of new cells infected at 5 days by approximately 10-fold versus untreated cells (Lischka *et al.,* 2010).

OTHER VIRUSES

Letermovir is inactive against all other viruses tested, including alpha-herpesviruses, non-HCMV beta-herpesviruses, gamma-herpesviruses, adenovirus, hepatitis B virus, hepatitis C virus, retroviruses, and orthomyxoviruses (Marschall *et al.*, 2011). The simple reason for this inactivity is that the letermovir is active against a protein group (the terminase subunit) that is not found in other known viruses (see section 3, Mechanism of drug action).

2b. Emerging resistance and cross-resistance

RESISTANCE MECHANISMS

Studies have shown that the HCMV terminase complex (which includes pUL56 and pUL89, and possibly the portal protein pUL104), which is the target for letermovir, has a low frequency of natural polymorphisms (interstrain identity > 97.7% at both nucleotide and amino acid levels), making it likely that most, if not all, natural strains of HCMV are susceptible to letermovir (Pilorgé *et al.*, 2014). Further, letermovir's mechanism of action (see section 3, Mechanism of action) is completely different from the mechanisms of existing nucleoside and nucleotide antivirals, such as ganciclovir and cidofovir (see Chapter 215, Ganciclovir and valganciclovir and Chapter 216, Cidofovir and brincidofovir) and also from direct polymerase inhibitors like foscarnet (see Chapter 219, Foscarnet). Consequently, cross-resistance with other, currently available HCMV antiviral drugs is highly unlikely. The unlikeliness of this has been substantiated by a case report showing that an HCMV strain resistant to ganciclovir, cidofovir, and foscarnet remained susceptible to letermovir (Kaul *et al.*, 2011).

Laboratory development of letermovir-resistant HCMV strains by serial passage in subinhibitory concentrations of letermovir as well as single-step resistance selection assays led to the creation of letermovir-resistant HCMV mutants harboring polymorphisms in the *UL56* gene, coding for a subunit of the HCMV terminase complex (Goldner *et al.,* 2014). After genetic recombination into the laboratory AD169 HCMV strain, *UL56* mutations V231L, V236M, R369M, R369G, and R369S lead to 5- to 50-fold increases in letermovir EC_{50}, while the L241P and C325Y mutations increased the EC_{50} 218- and 8,796-fold, respectively. In addition, the A345S polymorphism in the *UL89* gene was noted in two resistant isolates, although prior studies demonstrated that this polymorphism does not influence viral susceptibility to letermovir (Bradley *et al.*, 2009; Lischka *et al.*, 2010). Resistance-conferring mutations in other subunits of the terminase complex (i.e. *UL89, UL51*) or portal proteins (pUL104), were not observed (Goldner *et al.*, 2014; see Table 220.1). Studies by Goldner and co-workers (2011) showed that a single conservative L241P or R369S amino acid substitution was necessary and sufficient to produce letermovir resistance *in vitro.*

A separate serial-passage study of a derivative of the AD169 strain, T4138, re-created six of the seven resistance-conferring *UL56* mutations (Chou, 2015). Multiple novel polymorphisms in *UL56* were also identified, leading to variable fold changes in the letermovir EC_{50}. L51M, V231A, T244K, and F261L all led to less than 4-fold changes; V263L, E237D, L257I, F261C, Y231C, and M329T resulted in 4- to 15-fold increases; and C325F and C325R led to > 3000-fold increases in the EC_{50}. Several strains with multiple mutations were also derived. Excluding the high-level C325F/R resistance polymorphisms, multiple mutations conferred higher degrees of resistance than any individual polymorphism alone. Mutations generally appeared in a stepwise process, with the low-level resistance *UL56* polymorphism F261L appearing at low concentrations of letermovir and being replaced by polymorphisms conferring higher degrees of resistance at increasing letermovir concentrations. In comparison to foscarnet, mutations conferring resistance to letermovir appears at earlier stages of serial passaging and do not affect viral fitness, indicating that letermovir may have a lower genetic barrier to resistance than other anti-HCMV antivirals (Goldner *et al.*, 2014; Chou, 2015). Table 220.1 contains a list of identified *UL56* mutations and their associated resistance profile.

Multiple naturally occurring polymorphisms in *UL56* have been identified in isolates that have never been exposed to letermovir or other HCMV terminase inhibitors (e.g. benzimidazole D-ribonucleosides) although the region is highly genetically conserved among clinical isolates (Champier *et al.*, 2008; Pilorgé, *et al.*, 2014). The sensitivity of HCMV harboring two naturally occurring polymorphisms in a highly