Table 220.1. Genotype and *in vitro* phenotype of identified *UL56* polymorphisms.

UL56 amino acid substitution (genotype)	Letermovir EC_{50} (nM) (phenotype)	Fold change in EC_{50} vs. wild-type HCMV
C325F[a]	21,000	> 3,000
C325R[a]	20,000	> 3,000
C325Y[a]	20,000	> 3,000
D414N[b]	3	0.9
E237D[a]	58	10
F261C[a]	25	4.4
F261L[a]	16	2.8
LI34V/Q228H[b]	1.8	0.6
L241P[a]	550	96
L257I[a]	28	4.9
L51M[a]	4.3	0.8
M329T[a]	25	4.4
R369G[c]	60	13
R369M[c]	110	23
R369S[c,d]	370	81
R410G[b]	1.2	0.4
S227I[b]	0.6	0.2
T244K[a]	19	3.3
V231A[a]	12	2.1
V231L[a]	29	5.1
V236M[c]	90	19
V263L[a]	80	14
Y321C[a]	26	4.6

[a]Data from Chou (2015).
[b]Data from Lischka *et al.* (2016).
[c]Data from Goldner *et al.* (2014).
[d]Present with A345S mutation in *UL89*.

conserved region of the *UL56* gene (spanning AA 230–370) was assessed based on the known association of this gene with letermovir resistance (Goldner *et al.*, 2014; Goldner *et al.*, 2015). In comparison to a wild-type strain, both the D242G and A327V mutations were not found to confer resistance to letermovir, and in fact drug sensitivity was increased two- to threefold with no alterations in viral fitness (Goldner *et al.*, 2015).

EMERGENCE OF RESISTANCE

The impact of the unusually steep dose–response curve for letermovir *in vitro* has been suggested as a mechanism protecting against the development of resistance *in vivo,* presumably by reducing the dose window for active selection of resistance mutations (Lischka *et al.,* 2016). In contrast, studies by Chou (2015) have shown the rapid emergence of high-grade letermovir-resistant mutants in engineered HCMV viral strains, which may be interpreted as predictive of a low barrier to resistance.

Breakthrough HCMV infections while on letermovir were observed during the phase II trial of letermovir for the prevention of HCMV in hematopoietic cell transplant recipients (Chemaly *et al.*, 2014). The corresponding clinical HCMV isolates underwent focused sequencing of the *UL56* gene and were phenotypically characterized to assess for letermovir resistance (Lischka *et al.*, 2016). Six unique *UL56* mutations were identified in five isolates out of the 15. On phenotypic characterization, the observed L134V, Q228H, R410G, and D414N *UL56* mutations did not lead to decreased letermovir sensitivity (Lischka *et al.*, 2016). The S227I mutation led to a 5-fold increase in sensitivity to letermovir, in concordance with previously described findings that other mutations in the AA 230–370 region may be associated with hypersensitivity to letermovir (Goldner *et al.,* 2015; Lischka *et al.,* 2016). The previously characterized V236M mutation was found in only one patient who was receiving the lowest dose in the phase II study (60 mg/day; less than a quarter of the therapeutic dose being used for ongoing phase III trials, ≥ 240 mg/day); this isolate had a 46-fold increase in letermovir EC_{50} compared to wild-type virus in accordance with prior *in vitro* work (Goldner *et al.*, 2014; Lischka *et al.*, 2016). This patient was subsequently successfully treated with ganciclovir, indicating the lack of cross-resistance (Chemaly *et al.,* 2014). Further, no resistant HCMV strains have been seen in subjects in phase II or III studies receiving letermovir at doses of ≥ 240 mg/day, and achieved full suppression of breakthrough HCMV viremia in HSCT recipients (Lishka *et al.,* 2016).

CROSS-RESISTANCE

As letermovir and other licensed anti-HCMV antivirals have different target sites, cross-resistance between letermovir and other compounds is simply not expected. The ability of resistance polymorphisms to existing anti-HCMV DNA polymerase inhibitors to confer cross resistance to letermovir was assessed in 63 clinical HCMV strains from immunocompromised patients (Pilorgé, *et al.*, 2014). A total of 30 strains were resistant to ganciclovir, cidofovir, and foscarnet, alone or in combination. Polymorphisms in the *UL56, UL89*, and *UL104* genes were no more common among drug-resistant isolates than drug-sensitive isolates, indicating that resistance to DNA polymerase inhibitors does not lead to cross-resistance to letermovir. Mutations that confer resistance to other, structurally unrelated, inhibitors of the HCMV terminase complex (i.e. BAY 38-47766 and the benzimidazole D-ribonucleosides) are at distinct sites in the *UL56* gene and o not confer cross-resistance to letermovir (Goldner *et al.*, 2011). The activity of letermovir against HCMV isolates harboring various *UL97* and *UL54* mutations and in *vitro* resistance to ganciclovir and/or cidofovir was also confirmed against laboratory-derived isolates, with letermovir EC_{50} values ranging from 1.6 to 5.1 nM and at generally lower values than those observed in wild-type isolates (Lischka *et al.,* 2010; Marschall *et al.*, 2011). Two investigational benzimidazole ribonucleoside HCMV terminase inhibitors (BDCRB and TCRB) and the structurally distinct sulfonamides (e.g. BAY 38-4766) do not show cross-resistance with letermovir, indicating the exquisite specificity of that drug to pUL56 (Goldner *et al.,* 2011).