resistant to aciclovir, > 4800 more resistant to brivudin, and 13–32 times more resistant to foscarnet (foscarnet IC_{50} values were 41.9, 58.7, and 63.7 μM on initial isolation and 811, 1327, and 1321 μM after selection). Multiple open reading frames were assessed for changes by polymerase chain reaction (PCR) amplification and sequencing, and all strains had changes in the thymidine kinase and polymerase regions. The investigators found sox foscarnet-resistant mutations: five in the polymerase gene and one in thymidine kinase (L92M). The latter mutation was puzzling because foscarnet exposure should not exert pressure on the thymidine kinase (TK) gene.

Two HHV-6 strains made resistant to foscarnet by *in vitro* passage had four mutations in the virion polymerase, T435R, H507Y, C525S, and F292S. In a cell-free assay of the virion polymerase, each mutation increased foscarnet resistance (Bonnafous *et al.*, 2007). Known HHV-6 mutations mediating resistance to foscarnet are summarized in Table 219.5 (Piret and Boivin, 2014).

No strains of other herpesviruses resistant to foscarnet have been reported.

HUMAN IMMUNODEFICIENCY VIRUS

Strains of with reduced sensitivity to foscarnet have been identified by passage in culture in the presence of increasing concentrations of the drug and from a patient with AIDS on long-term foscarnet therapy (Mellors *et al.*, 1995; Tachedjian *et al.*, 1995). It is interesting that foscarnet-resistant strains of HIV also showed impaired fitness (Tachedjian *et al.*, 1998b). Mutations in three reverse transcriptase codons (E89K, L92I, and S156A) and in codons W88S/G, Q161L, and H208Y, were also found, and their role in foscarnet resistance was confirmed by site-directed mutagenesis (Mellors *et al.*, 1995; Tachedjian *et al.*, 1995; Tramontano *et al.*, 1998). Other investigators have described foscarnet-resistant reverse transcriptase enzymes with mutations in codons V90A/T/G and E89G that were cross-resistant to the active metabolites of zalcitabine, didanosine, and zidovudine but that, at least in the case of the codon 90 mutation, remained susceptible to nevirapine and other nonnucleoside reverse transcriptase inhibitors (Prasad *et al.*, 1991; Im *et al.*, 1993). Nakano *et al.* (1997) used clonal selection and molecular evolutionary techniques to identify foscarnet-resistant strains of HIV-1 due to the unique reverse transcriptase mutation R172K.

Table 219.5. Polymerase (*U38* gene) mutations thought to mediate resistance of HHV-6 to foscarnet and other antiviral drugs.

Mutation(s)	Resistance to other drugs	References
F292S		Bonnafous *et al.* (2007)
T435R		Bonnafous *et al.* (2007)
H507Y		Bonnafous *et al.* (2007)
C525S		Bonnafous *et al.* (2007)
R798I	GCV, CDV	Piret and Boivin (2014)
A961V (*U69* gene) + M138V (*U38* gene)	GCV, CDV	Piret and Boivin (2014)

Abbreviations: HHV: human herpesviruses; GCV: ganciclovir; CDV: cidofovir.

Foscarnet-resistant strains of HIV have been shown by several investigators to be hypersusceptible to zidovudine and nevirapine, with unaltered sensitivity to didanosine and zalcitabine (Mellors *et al.*, 1995; Tachedjian *et al.*, 1995; Smith *et al.*, 2006). Mutations that confer foscarnet resistance can suppress zidovudine resistance (Tachedjian *et al.*, 1996); as a consequence, HIV-1 variants resistant to both foscarnet and zidovudine are uncommon. In the extremely rare patients who are given long-term combination therapy with these drugs, the resulting dually resistant viruses have multiple reverse transcriptase mutations (Tachedjian *et al.*, 1998a). Conversely, viruses resistant to zidovudine, and those with multiple thymidine analog mutations (TAMs), were hypersusceptible to foscarnet (Tachedjian *et al.*, 1996; Mathiesen *et al.*, 2007); these *in vitro* data were supported by *in vivo* studies showing that foscarnet therapy of HIV infection was more effective against HIV strains having more than three preexisting TAMs than it was against strains with fewer TAMs (Charpentier *et al.*, 2008).

Some elements of the interaction between foscarnet and chain-terminating nucleoside analogs such as zidovudine are now understood. The HIV reverse transcriptase can unblock a chain-terminated DNA strand by phosphorolytic cleavage of the chain-terminating nucleotide (e.g. zidovudine monophosphate). Reverse transcriptase mutations mediating zidovudine resistance increase phosphorolytic (unblocking) activity, whereas mutations mediating foscarnet resistance decrease it (Meyer *et al.*, 2003).

Zidovudine and didanosine mutants of feline immunodeficiency virus that were selected in culture showed cross-resistance to foscarnet and increased sensitivity to zalcitabine (Gobert *et al.*, 1994).

2c. *In vitro* synergy and antagonism

A number of *in vitro* studies have found additive or synergistic activity of foscarnet with other antiviral drugs, including those with the same target as foscarnet (viral DNA polymerases), and against viruses otherwise susceptible to foscarnet.

Synergistic inhibition of replication of CMV *in vitro* by combinations of ganciclovir and foscarnet has been reported (Manischewitz *et al.*, 1990). Combinations of foscarnet and trifluorothymidine have also been shown as synergistic (Spector *et al.*, 1983). Likewise, work in Hirsch's laboratory suggested that combinations of foscarnet with most CMV-active antiviral drugs would be either additive or synergistic (Manion *et al.*, 1996). Although a review of clinical studies showed additive or synergistic interactions between foscarnet and ganciclovir (consistent with previous studies), but there was only a suggestive clinical benefit for combination therapy with foscarnet (Drew *et al.*, 2006). Although additive or synergistic antiviral effects against CMV have been observed when combining foscarnet with ganciclovir or cidofovir (Manion *et*