et al., 1999). Assays based on quantitation of fluorescent cells, DNA, or viral antigen in a different cell line gave EC_{50} values of 4–10 μM (Williams *et al.*, 2003). These antiviral potencies are similar or superior to that of aciclovir when tested in parallel.

OTHER VIRUSES

Maribavir was tested in cell culture against a variety of animal and human herpesviruses (Williams *et al.*, 2003) and no antiviral activity was detected against herpes simplex, varicella-zoster, human herpesvirus 6, human herpesvirus 8, and several animal cytomegaloviruses. The U69 kinase of human herpesvirus 6 appears to be inhibited by maribavir, but viral replication was not meaningfully inhibited in proliferating cells (Prichard *et al.*, 2011).

2b. Emerging resistance and cross-resistance

Serial propagation of CMV in cell culture with increasing concentrations of maribavir results in mutations of the viral *UL97* kinase gene (Biron *et al.*, 2002; Chou *et al.*, 2007; Chou and Marousek, 2008; Chou *et al.*, 2012) and the *UL27* gene (Komazin *et al.*, 2003; Chou *et al.*, 2004; Chou, 2009). *UL97* mutations V353A, L397R, T409M, and H411L/N/Y individually confer moderate to high-level resistance to maribavir (9- to > 200-fold increases in EC_{50}), and combinations of mutation V353A with one of those at codons 409 or 411 confer > 150-fold increases in EC_{50} (Chou *et al.*, 2007; Chou and Marousek, 2008). These mutations cluster at the conserved ATP binding region of the *UL97* kinase and probably impair the binding of maribavir as an inhibitor (Chou and Marousek, 2008). Diverse *UL27* mutations, including stop and frameshift mutations predicting loss of function, confer low-grade maribavir resistance (2- to 5-fold increases in EC_{50}), probably by altering a function that is unfavorable in the absence of *UL97* kinase activity because *UL27* mutations spontaneously develop during propagation of *UL97* kinase knockout strains (Chou, 2009). Recent studies suggest that *UL27* normally acts to upregulate the cyclin-dependent kinase inhibitor p21(Cip1) (Reitsma *et al.*, 2011; Bigley *et al.*, 2015). *UL27* and *UL97* mutations may combine to confer a higher overall level of maribavir resistance (Chou *et al.*, 2012).

In two clinical case reports (Strasfeld *et al.*, 2010; Schubert *et al.*, 2013), the use of maribavir as salvage therapy of CMV infection with high circulating viral loads resulted in the relatively rapid emergence (after 1–2 months) of the same *UL97* mutations T409M, H411Y, and H411N. Salvage therapy in 35 subjects resulted in five more maribavir-resistant CMV strains, all involving *UL97* mutations T409M or H411Y (Alain *et al.*, 2015). While T409M and H411Y/N are emerging as the most common maribavir resistance mutations in clinical practice, the number of cases is still too small to assess the incidence of resistance in various treatment settings. No UL27 maribavir resistance mutations have yet been documented in clinical specimens, and no genotypic evidence of maribavir resistance was noted in prophylaxis trials (Chou *et al.*, 2012).

Because of different mechanisms of action, cross-resistance was not expected with maribavir and existing CMV DNA polymerase inhibitors (ganciclovir, foscarnet, and cidofovir) (McSharry *et al.*, 2001; Biron *et al.*, 2002; Drew *et al.*, 2006) as well as letermovir (Goldner *et al.*, 2011). *UL54 pol* gene mutations that confer resistance to polymerase inhibitors have no effect on maribavir susceptibility (Drew *et al.*, 2006). *UL97* mutations that emerge after exposure to ganciclovir preferentially involve codons 460, 520, or 590–607 and show no maribavir cross-resistance (Drew *et al.*, 2006; Lurain and Chou, 2010; Shannon-Lowe and Emery, 2010), while *UL97* mutants selected after maribavir exposure as listed earlier remain susceptible to ganciclovir (Chou and Marousek, 2008). However, some atypical *UL97* mutations can confer dual maribavir and ganciclovir resistance. Mutations that knock out *UL97* kinase activity (e.g. involving the critical residue K355) inherently confer resistance to both drugs because the phosphorylation of ganciclovir is impaired and there is no kinase activity left to be inhibited by maribavir (Chou *et al.*, 2013). Such mutants are severely growth impaired and have not been authenticated in clinical specimens. Of greater interest is a p-loop mutation F342S in the *UL97* ATP binding domain, selected *in vitro* by serial passage with the nucleoside analog cyclopropavir, which confers ganciclovir and maribavir cross-resistance without major growth impairment (Chou *et al.*, 2013). Such p-loop mutations have not yet been reported in clinical specimens but are not looked for in the current genotypic resistance assays for ganciclovir resistance.

2c. *In vitro* synergy and antagonism

Maribavir is expected to antagonize the antiviral action of ganciclovir by blocking the initial *UL97*-mediated phosphorylation of ganciclovir that is necessary for its antiviral action. This antagonism has been experimentally demonstrated by checkerboard assays and fractional inhibitory concentrations (Chou and Marousek, 2006), but was not detected in earlier studies (Evers *et al.*, 2002; Selleseth *et al.*, 2003) probably because of differences in cell cultures and assay methods. Direct biochemical evidence that maribavir blocks ganciclovir phosphorylation in infected cells has not been published, but current information suggests that combined therapy with maribavir and ganciclovir is inadvisable.

Maribavir–foscarnet and maribavir–cidofovir combinations showed neither synergy nor antagonism by checkerboard assays (Chou and Marousek, 2006). This is compatible with the finding of an additive interaction of the same drug pairs when analyzed using a different method (Selleseth *et al.*, 2003), and different from the finding of synergy in a third study (Evers *et al.*, 2002). Overall data indicate that maribavir may be combined with either foscarnet or cidofovir without concern for therapeutic antagonism.

3. MECHANISM OF DRUG ACTION

Maribavir is a specific ATP-competitive inhibitor of the CMV *UL97* kinase, active at nanomolar concentrations in assays