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## Mutation of the Thymidine Kinases Encoded by Herpes Simplex Virus or Vaccinia Virus can Confer Resistance to 5-Iodo-4′-thio-2′-deoxyuridine

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Recent reports have shown that 5-iodo-4′-thio-2′-deoxyuridine (4′-thioIDU) inhibits the replication of orthopoxviruses both in vitro and in vivo. We have also shown that this molecule has broader antiviral activity and inhibited the replication of some herpesviruses including herpes simplex virus (HSV), varicella-zoster virus, and human cytomegalovirus. Previous studies with this compound showed that it inhibited viral DNA synthesis at a concentration similar to that required to inhibit viral replication and suggested that this inhibition was responsible for its antiviral activity. A drug resistant virus was selected by the serial passage of vaccinia virus under the selective pressure of the compound. An isolate was obtained that exhibited a 10-fold decrease in its susceptibility to the compound, but was fully susceptible to other inhibitors, such as ST-246 and CDV. The resistant virus had a 3 aa deletion in the thymidine kinase (TK) open reading frame (ORF) and confirmed that the enzyme was important for the activity of the compound. No mutations were detected in the DNA polymerase, but these data do not exclude the possibility that mutations in this ORF might also confer resistance to 4′-thioIDU. A similar strategy was employed to obtain a drug resistant isolate of the MS strain of HSV-2. The resulting isolate was highly resistant to 4′-thioIDU and had a frameshift mutation in the TK ORF resulting in a truncated protein. These results suggest that the TK homologs encoded by HSV-2 and vaccinia virus may both be involved in the phosphorylation of the compound and suggest that it inhibits both viruses by a similar mechanism. While the DNA polymerases of both viruses are likely the ultimate target of 4′-thioIDU metabolites, mutations in the TK homologs appear to be important in the development of resistance to this compound.

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## Conserved Retinoblastoma Protein-binding Motifs in Human Cytomegalovirus UL97 Minimally Impact Viral Replication But can Affect Susceptibility to Maribavir

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The human cytomegalovirus (HCMV) UL97 protein kinase phosphorylates ganciclovir, but has also been shown to phosphorylate and inactivate the retinoblastoma tumor suppressor protein (Rb). This activity is significant because many viruses including adenoviruses, papillomaviruses and polyomaviruses share this activity and is thought to contribute to their ability to transform cells. We recently reported that the inhibition of UL97 kinase activity by maribavir (MBV) can inhibit the inactivation of Rb by the UL97 kinase and is an interesting aspect of its antiviral activity. To assess the impact of Rb inactivation on the antiviral activity of MBV, recombinant viruses were constructed with mutations in each of the three consensus Rb-binding motifs and all were evaluated for their susceptibility to the drug. All of these viruses replicated well in human foreskin fibroblasts with only a slight delay in replication kinetics. These viruses also remained fully susceptible to cidofovir and ganciclovir, but mutation of the amino terminal Rb-binding motif rendered the virus modestly hypersensitive to MBV. This result was confirmed by constructing another recombinant virus with a similar mutation in this same motif, which also imparted hypersensitivity to MBV. Interestingly, this was also the only motif that appeared to impact the phosphorylation of Rb in infected cells. These studies suggest that the disruption of individual motifs did not have major effect on viral replication. However, the disruption of the amino terminal Rb-binding motif increased susceptibility to MBV and suggested that this activity was related to the mechanism of action of the drug. The UL97 kinase plays a critical role in the therapy of HCMV infections because it phosphorylates ganciclovir but equally important is its inhibition by MBV, which represents a new strategy for the therapy of viral infections. Clinical trials with this drug will help validate this new approach and will improve our understanding of UL97 kinase function in infection.

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## Antiviral Activities of Nucleoside Analogs Against HSV-1 Replication in 143B Cells Expressing the Viral TK Genes with Different Initiation Codon Sites

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The thymidine kinase (TK) gene of HSV-1 has several initiation codons and the polypetides with different sizes are usually observed in Western analysis of virus-infected cell lysates.. To confirm whether smaller TK proteins have functions for antiviral activation, we have constructed plasmids for eukaryotic expression of the TK gene of HSV-1 strain F: transcribed from 1st initiation codon (F1TK) (376 amino acids, 41.0 kDa), from 2nd codon at 46 M (F2TK) (331 amino acids, 35.7 kDa) and from 3rd codon at 60 M (F3TK) (317 amino acids, 34.1 kDa). Cellular TK-deficient 143B cells were transfected with them by calcium phosphate-DNA coprecipitation method and neomycin-resistant cells were selected. The TK gene expressing cell lines were further cloned (more than two clones per plasmid construct) and antiviral evaluation with them was performed. Acyclovir and ganciclovir gained their antiviral activity against TK-deficient AR1 and AR3 in the F1TK143B and F2TK143B but not in F3TK143B cells. (E)-5-(2-bromovinyl)-2′-deoxyuridine did not show increased antiviral activities in all of them. The TK polypeptides in the F3TK143B was hardly detected in immunofluorescence confocal microscopy. It should be studied further whether the smaller TK proteins have special functions in HSV pathogenesis.

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