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### The Combination of Anti-poxvirus Compounds ST-246 and TTP-018 are Synergistic In Vitro

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ST-246 and TTP-018 are low molecular weight compounds that inhibit orthopoxvirus replication through distinct mechanisms of action (Yang et al., 2005; Bolken et al., 2006). The antiviral effects of each compound alone or in combination were evaluated in vitro using drug–drug combination analysis. Using two mathematically robust techniques (Loewe Additivity and Bliss Independence null reference models of additivity) to analyze the experimental data, significant synergistic effects were observed resulting in a decrease in the EC50 value for ST-246 and TTP-018 of 5-fold and 7.5-fold, respectively. The combination index (CI) was found to be 0.47 indicating a synergistic interaction between the two compounds. Evaluation of the data using a three-dimensional dose response model (MacSynergy II program) generated a synergy volume > 50 unit 2% at the 95% confidence level, implying moderate synergy with potential importance in vivo. Both analyses confirmed the synergistic interaction of ST-246 and TTP-018. In addition, there was no evidence of cytotoxicity with any of the compounds alone or in combination at the concentrations tested. Our findings suggest that the combination of ST-246 and TTP-018 produce greater than additive or synergistic antiviral effects in vitro.

### References

Bolken, et al., 2006. Abstract in 2006 FASEB Summer Research Conference Poxviruses.
Yang, et al., 2005. J. Virol. 79, 13139–13149.

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### Thiazolobenzimidazoles, a Novel Class of Enterovirus Inhibitors, Target the 2C Protein

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Despite the fact that enteroviruses are implicated in a variety of human diseases, there is no approved therapy for the treatment of enteroviral infections. We previously reported on a series of 2,6-dihalophenyl-substituted 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles with anti-enterovirus activity. In order to unravel the mechanism of action of these compounds, time-of-drug addition assays were performed, and virus, resistant to the most potent compound (CHI-033) was generated. Genotyping of drug-resistant strains revealed four amino acid mutations in protein 2C: K107R, A224V, I227V and A229V. Mutations at the latter two positions have been described earlier for echoviruses (Klein et al., 2000) that are resistant to [2-(*a*-hydroxybenzyl)-benzimidazole] (HBB), which suggests a similar mechanism of action Moreover, poliovirus, resistant to guanidine hydrochloride, another 2C inhibitor has been reported to carry mutations at positions 225 and 227 (Pincus et al., 1986). This suggests an important role for the 2C region encompassing amino acids 224–229 in enteroviral replication. To study whether or not individual mutations are sufficient to confer resistance, either single mutations or multiple mutations are being introduced in a full-length infectious clone of coxsackievirus B3. (Cross)-resistance profiles will be determined with the selected 2C inhibitors as well as for other known enterovirus inhibitors. It is our aim to unravel the precise molecular mechanism by which these compounds inhibit the function of 2C and thus viral replication.

### References

Klein, et al., 2000. J. Gen. Virol. 81, 895–901.
Pincus, et al., 1986. J. Virol. 57 (2), 638–646.

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### Selective Phosphorylation of Antiviral Drugs by Vaccinia Virus Thymidine Kinase

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The antiviral activity of a new series of thymidine analogs was determined against vaccinia virus (VV), cowpox virus (CV), herpes simplex virus, and varicella zoster virus. Several compounds were identified that had good activity against each of the viruses tested including (*N*)-methanocarbathymidine, and a series of 5-substituted thymidine analogs. To investigate the possibility that these drugs might be phosphorylated preferentially by the viral TK homologs, the antiviral activity of these compounds were also assessed using TK negative strains of some of these viruses. Some of these compounds were shown to be much less effective in the absence of a functional TK gene in CV, which was unexpected given the high degree of homology between this enzyme and its cellular homolog. This unanticipated result suggested

that the CV TK was important in the mechanism of action of these compounds and also that it might phosphorylate a wider variety of substrates than other type II enzymes. To confirm these data, we expressed the VV TK and human TK1 in bacteria and isolated the purified enzymes. Enzymatic assays demonstrated that the viral TK could efficiently phosphorylate many of these compounds, whereas most of the compounds were very poor substrates for the cellular kinase, TK1. Thus, the selective phosphorylation of these compounds by the viral kinase may be sufficient to explain the TK dependence. This unexpected result suggests that selective phosphorylation by the viral kinase may be a promising new approach in the discovery of highly selective inhibitors of orthopoxvirus infections.

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## Intracellular Localization of Herpes Simplex Virus Type 1 Thymidine Kinase in Cells Infected with Various Thymidine Kinase-Deficient Viruses

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The intracellular localization of the viral thymidine kinase (TK) in HSV-1-infected cells using immunofluorescence confocal microscopy was a powerful tool to confirm whether virus entered its host cells. The TK polypeptides were detected strongly in the nucleus of Vero cells from 2 h p.i. to the end of viral growth in one-step growth experiments. Localization of herpes TKs fused with green fluorescent protein in TK-transfected human osteosarcoma cells deficient in cytosolic TK showed HSV-1 TK in the nucleus and multiple nuclear localization signals in the HSV-1 TK proteins were proposed by Delgre've et al. (1998 and 1999): $^{25}$RRTALRPRR$^{33}$, R236–R237 and K317–R318. We have isolated several drug-resistant TK-deficient mutants by treating virus-infected cells with acyclovir or penciclovir: AR1 ∼ AR9 and PR1 ∼ PR5. They showed the mutation(s) in the TK gene leading amino acid substitution(s), prematuration or frame-shift prematuration or elongation of the polypeptides. They were classified into 2 groups, viz. the TK-partial (TK$^{p}$) and TK-negative (TK$^{-}$) groups, by measuring their enzyme activity.

We have examined the intercellular TK localization profiles of the TK-mutnats by fixing virus-infected cells and probing with rabbit anti-TK antisera and FITC-anti-rabbit IgG antibodies. There were strong correlation between of the TK amount appeared in the Western data and localization data. Most of the TK$^{-}$ mutants showed little fluorescence and most of the TK$^{p}$ showed strong fluorescence both in the nucleus and the cytoplasm although there were few exceptions. It also looked like the amount of the TK proteins detected were profoundly affected by the presence of the nuclear transport signals.

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## Substrate Specificity and Molecular Modelling of Feline Herpesvirus-1 Thymidine Kinase

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Feline herpesvirus-1 (FHV-1) causes severe upper respiratory and ocular disease in cats. Despite routine vaccination, FHV-1 continues to be a clinical problem. The similarities between the ocular diseases caused by FHV-1 and HSV-1, encouraged the use of acyclovir (ACV) to treat FHV-1 infections in cats. However, virological data and some clinical studies have cast doubt on its efficacy. The objectives of this work were to investigate the mechanisms of the relative insensitivity of FHV-1 to ACV and to develop alternative effective candidates based on the various anti-herpetic nucleoside analogues that are currently used in humans.

Although FHV-1 is normally insensitive to ACV, it was shown to acquire sensitivity in a transformed feline cell line expressing the HSV-1 thymidine kinase (TK). The $IC_{50}$ value of ACV for FHV-1 was markedly reduced from 110 μM to 0.1 μM in the HSV-1 TK-transformed cells. This result has suggested that inefficient phosphorylation by the FHV-1-encoded TK was responsible for the relative insensitivity of this virus to the antiviral action of ACV.

Using recombinant enzymes expressed in *E. coli*, the substrate specificity of FHV-1 TK was demonstrated to be relatively limited when compared with that of its HSV-1 counterpart. FHV-1 TK efficiently phosphorylated its natural substrate deoxythymidine, but exhibited a relatively low affinity for the guanosine analogue substrates. PCV was the most efficiently phosphorylated (2287 pmoles/min), followed by GCV with a two-fold reduction in the phosphorylation rate (1067 pmoles/min). ACV was a very poor substrate for the FHV-1 TK with a rate of 236 pmoles/min.

To correlate biochemical data with structural features, computational homology modelling techniques were used to generate a model for the 3D structure of FHV-1 TK. Site-directed mutagenesis experiments verified this model. Based on these analyses, the structural constraints of the FHV-1 TK active site responsible for the poor ACV phosphorylation were identified. It was also possible to explain the ability of FHV-1 TK to preferentially phosphorylate PCV by the structural flexibility of this substrate within the active site.

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