

and reduced the accumulation of viral DNA by less than 10-fold at 10 μ M. Combinations of CMX001 and GCV were synergistic, when concentrations of CMX001 as low as 3 picomolar were added to GCV. No significant changes in cytotoxicity were observed for any of the concentrations tested confirming that the combination was not toxic. The exceptional potency of CMX001 observed in these assays was confirmed in a quantitative real-time RT-PCR-based array that determined levels of all viral transcripts. Reductions in the levels of viral transcripts were consistent with the reductions in genome copy number and reflected the marked inhibition of viral replication in vitro relative to GCV. These results clearly indicated that combinations using suboptimal concentrations of CMX001 with GCV are synergistic in vitro. In vivo studies should be performed to further explore this combination, especially in drug resistant HCMV.

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In Vivo Efficacy of Twice Daily Oral Treatment with N-MCT Against Herpes Simplex Virus Type 2 in Balb/C Mice

Debra Quenelle^{1,*}, Robert Glazer², Aquilur Rahman², Deborah Collins¹, Terri Rice¹

¹ The University of Alabama School of Medicine, Birmingham, USA; ² N & N Scientific, Inc., Rockville, USA

N-MCT has been previously reported to have excellent activity both in vitro and in vivo against human herpesviruses (HSV). Mice were lethally infected intranasally with herpes simplex virus (HSV), type 2, strain MS and treatments were initiated 24, 48 or 72 h post-viral infection. Compound was suspended in 0.4% carboxymethylcellulose to yield desired dosages in a 0.2 ml volume. N-MCT was administered orally twice daily at 100, 50 or 25 mg/kg and continued for 7 days. Acyclovir (ACV) was given similarly at 100 mg/kg as a positive control. No toxicity was observed in uninfected mice treated with N-MCT. All dosages of N-MCT were highly effective ($p < 0.001$) in reducing mortality when treatments were initiated 24, 48 or 72 h post-viral infection. Pathogenesis studies were performed to determine the effect on viral replication in target organs of lung, liver, spleen, kidney and sections of brain. Treatment with N-MCT significantly reduced viral replication of HSV-2 better than ACV in all organs, particularly within the CNS. In order to determine the lowest effective dose, additional studies using lower doses were performed with N-MCT from 25 to 0.01 mg/kg given twice daily beginning 24 h post-viral inoculation. All doses of N-MCT greater than 0.03 mg/kg significantly reduced mortality ($p < 0.001$). The lowest dose evaluated was 0.01 mg/kg which also reduced mortality to 40% ($p = 0.001$) and increased the mean day to death from 7.5 to 10 days ($p = 0.001$). N-MCT is a potent antiviral for herpesvirus and has potential for the treatment of serious HSV type 1 and 2 infections in humans.

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Enhanced Efficacy Using Combinations of CMX001 with Acyclovir Against Herpes Simplex Virus Infections In Vitro and In Mice

Debra Quenelle^{1,*}, Mark Prichard¹, Carroll Hartline¹, Emma Harden¹, Deborah Collins¹, Terri Rice¹, Randall Lanier², Earl Kern¹

¹ The University of Alabama School of Medicine, Birmingham, USA;

² Chimerix, Inc., Durham, USA

Previous studies have shown that either CMX001 (HDP-cidofovir) or acyclovir (ACV) is effective in vitro against herpes simplex virus (HSV) isolates and in preventing mortality of mice infected intranasally with HSV-1 or 2. Evaluation of efficacy using suboptimal doses of these two agents in combination has not been previously reported. In cell culture, CMX001 was evaluated against a panel of both wild-type and ACV-resistant isolates of HSV-1 and HSV-2 and found to be highly effective with EC₅₀ values ranging from 0.008 to 0.03 μ M. These same virus isolates were also inhibited by concentrations of ACV ranging from 2.0 to >100 μ M. Using various concentrations of CMX001 and ACV in combination in tissue culture, we demonstrated synergistic efficacy without an increase in toxicity in cell culture experiments. To determine if this combination would result in enhanced efficacy in an animal model, CMX001 was given once daily at 1.25, 0.42 or 0.125 mg/kg with or without ACV to mice infected intranasally with HSV-2. ACV was given twice daily at 30, 10 or 3 mg/kg. Treatments were initiated 72 h post-viral infection by oral gavage for 7 days. As expected from previous work where 5 mg/kg was an optimal dose of CMX001 in this model, CMX001 as a single therapy at 1.25, 0.42 or 0.125 mg/kg did not significantly improve survival or increase the mean day to death (MDD). ACV alone improved survival at 30 mg/kg ($p = 0.06$) and significantly increased the mean day to death at 30 or 10 mg/kg ($p < 0.01$), but not at 3 mg/kg. Suboptimal doses of CMX001 and ACV given together significantly enhanced protection from mortality or increased the MDD when compared with either drug alone in 8 of 9 combination groups. No additive toxicity was detected. Our results indicate that low dose combinations of these two agents act synergistically in vitro and in vivo and should be considered for use in herpesvirus infections in humans.

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First Diastereoselective Synthesis of Pronucleotides

Edwain Rios Morales^{1,*}, Cristina Arbelo Roman¹, Jan Balzarini², Chris Meier¹

¹ Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Hamburg, Germany; ² Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

Pronucleotides represent a promising alternative to improve the biological activity of nucleoside analogs in antiviral and cancer chemotherapy. Two of the most successful prodrug-systems are the cycloSal-pronucleotides (Meier, 2002) as well as the nucleoside arylphosphoramidates (Congiatu et al., 2006). As the first approach is based on a selective chemical hydrolysis, the second technology requires an enzyme-mediated activation. Due to their synthesis pathways, so far derivatives belonging to these two classes of pronucleotides were obtained as 1:1 mixtures of diastereomers