

POSTER 14**THE MECHANISM OF ACTION OF GANCICLOVIR DEPENDS ON THE NATURE OF THE TARGETED VIRAL POLYMERASE**

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Infection with the human cytomegalovirus (HCMV) remains an important health problem in immunocompromised persons. Several drugs that target the viral DNA polymerase (UL54) have been developed to treat the infection. Ganciclovir (GCV), or its prodrug valganciclovir (VGCV), are acyclic nucleoside analogue inhibitors with an acyclic sugar moiety that contains the structural equivalent of a 3'-hydroxyl group. However, the detailed mechanism of action remains elusive, although previous data with the related herpesvirus polymerase suggested that the incorporated inhibitor interferes with the incorporation of nucleotides at position n+1 and n+2. Detailed structural and biochemical studies with the HCMV DNA polymerase (UL54) are hampered by difficulties to obtain this enzyme in large quantities. The crystal structure of the related RB69 DNA polymerase (gp43) is often used as a model system to explain mechanisms of inhibition of DNA synthesis and drug resistance. However, here we demonstrate that gp43 is approximately 300-fold less sensitive to GCV, when compared with UL54. In an attempt to identify major structural determinants for drug activity, we replaced critical regions of the nucleotide binding site of gp43 with equivalent regions of the HCMV enzyme and show that chimeric gp43-UL54 enzymes are resensitized against GCV. Helices N and P are identified as major determinants for drug binding and activity. Incorporation of the natural nucleotide at position n+1, following the incorporated nucleotide analogue, is severely compromised. We further demonstrate that HIV-1 reverse transcriptase (RT) is likewise inhibited by GCV; however, in this case, the enzyme is capable of incorporating three more nucleotides downstream of the GCV-terminated primer and DNA synthesis is ultimately inhibited at position n+3. Thus, GCV acts through different mechanisms on UL54 and HIV-1 RT, respectively. On UL54, GCV appears to act predominantly as a non-obligate chain-terminator, and on HIV-1 RT the incorporated inhibitor acts predominantly as a delayed chain-terminator. The specific interaction between inhibitor and enzyme can determine the mechanism of action.