

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-304

MICROBIOLOGY REVIEW(S)

**MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)**

NDA 21-304 (Original)

Reviewer: N. Biswal

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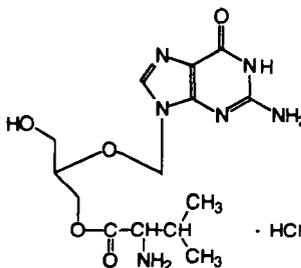
Sponsor: Syntex (USA) LLC.
3401 Hillview Av
Palo Alto, CA 94304

Product Names:

Code Names: _____^M, Valganciclovir hydrochloride, Ro-107-9070/194

Chemical Name: L-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9yl)methoxy]-3-hydroxypropyl ester, monohydrochloride

Structural Formula:



**APPEARS THIS WAY
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Molecular Formula: C₁₄H₂₂N₆O₅ •HCl

Molecular Weight: 390.83

Drug Category: Antiviral

Dosage Form/Route of Administration: 450 mg tablets/Oral

Indication: Treatment of Cytomegalovirus (CMV) retinitis in patients with AIDS

Supporting Documents: IND 48,106, NDA 25-082, NDA 19-661, NDA 20-460

INTRODUCTION

Ganciclovir (GCV) is an acyclic nucleoside analog of 2'-deoxyguanosine known to inhibit the replication of a number of human herpesviruses *in vitro* and *in vivo*. An intravenous (IV) formulation of GCV was first approved for the treatment of human cytomegalovirus (CMV) retinitis in immunocompromised patients (in June, 1989) and for the prevention of CMV disease in transplant patients (in May, 1992). Subsequently, an oral formulation of GCV was approved as an alternative to the IV formulation for maintenance treatment of CMV retinitis in patients with AIDS, whose retinitis has been stabilized by prior anti-CMV therapy. It was also approved for the prevention of CMV disease in solid organ transplant recipients, and in patients with advanced HIV infection at risk of developing CMV disease. However, oral GCV has a low bioavailability in humans (approximately 6%), thus limiting its clinical use.

To improve the bioavailability of GCV and to avoid the need for the intravenous administration of GCV, valganciclovir (VGCV), a valyl ester prodrug that is converted into GCV on absorption was developed. The antiviral properties of VGCV are stated to be those of GCV itself and oral administration of VGCV may provide GCV exposures equivalent to those achieved only with the IV formulation. Therefore, the sponsor is currently seeking marketing approval of a 450 mg tablet formulation of VGCV for the treatment of CMV retinitis in patients with AIDS. A total of 243 published and unpublished reports are enclosed in this submission documenting the preclinical and clinical antiviral activities of GCV. Most of these reports have been reviewed earlier for previous NDAs submitted for GCV IV and oral formulations.

Biology of CMV

CMV is a member of Herpesviridae family capable of causing a variety of acute, latent and recurrent infections in man and animals. Widespread in nature, these viruses are highly species specific, share common slow growth characteristics, and have a cytopathology involving nuclear and cytoplasmic inclusions. Human CMV, designated as the human herpesvirus 5, is the prototype of the betaherpesvirus group.¹ It has developed a peculiar parasitic relationship with human beings and in vast majority of cases human CMV infections are subclinical. However, immunodeficiency or immunosuppressive conditions predispose patients to acute human CMV infection or to reactivation of latent infection usually resulting in sight- and/or life-threatening diseases. In addition, congenital CMV infections are now the leading causes of mental retardation and birth defects in neonates.²

Like other herpesviruses, human CMV is able to establish latent infections with subsequent recurrence. Recurrent infection may result from (1) a low level chronic infection established after primary infection, (2) reinfection with a genetically diverse second strain of human CMV, or (3) repeated reactivation from latent state in response to different stimuli. It is not clear how the virus escapes from its latent state to initiate a variety of complex pathogenic processes in different organs of their otherwise symbiotic hosts.³

The ability of human CMV to infect a number of different cell types in the human host is not reflected, however, in tissue culture *in vitro* where it can efficiently replicate only in human diploid fibroblasts. Animal models such as CMV of mice, rat and guinea pig have become very useful for investigation of the intricate molecular mechanism(s) of CMV latency, including establishment, maintenance, and disruption of the latent state to reactivate infectious virus under various experimental conditions. It has been shown that both primitive and lineage-committed haemopoietic cells, particularly a small percentage of myeloid and dendritic cell progenitors, may act as a long-lived reservoir of latent human CMV. During latency, productive gene expression is shut down in

naturally infected hosts, and CMV gene expression has been shown to be restricted to transcripts from the immediate early (*ie1/ie2*) α -gene region. These cells can be reactivated by factors associated with haemopoietic cell differentiation and the inflammatory response.

The Virion

Human CMV, the largest member of human herpesvirus family, is approximately 200 nm in diameter. It consists of a 64 nm core enclosed by a 110 nm icosahedral capsid made up of 162 capsomeres.⁴ The complete virion is enveloped and is composed of about 30 structural proteins some of which are glycosylated. Three types of virus particles have been observed in human CMV-infected cells. Besides the usual infectious virions, infected cells also produce an equal number of noninfectious dense bodies composed largely of tegument protein pp65 but lacking nucleocapsids and viral DNA. The third type includes the noninfectious enveloped particles lacking an electron-dense DNA core.

The genome of human CMV is a double-stranded DNA of about 240 kb pairs in size.⁵ It consists of two unique, long (L) and short (S), covalently linked sequences, each bounded by inverted repeats. Inversion of the L and S segments leads to the production of four isomers of the viral DNA molecule. No other animal CMV genomes invert although some of them (e.g., equine and guinea pig CMV) have significant repeat sequences in their genome. The significance of the repeated sequences and genome inversion to the biology of CMV remains to be elucidated. The human CMV (AD169 strain) genome has been completely sequenced (EMBL accession number X17403).

Human CMV is capable of producing both productive (permissive) and abortive infections as well as transformation of cells cultured *in vitro*. Productive infection is initiated by attachment of the virion to specific receptors on a susceptible host cell. The virion envelope fuses with the plasma membrane or an endosome membrane and the naked capsid is transported to the nuclear pore to release the viral DNA in the nucleus. Several forms of intracellular CMV genome have been described, i.e., circular, concatemeric and linear double-stranded molecules. Viral DNA is transcribed in the nucleus throughout most of the replicative cycle. However, the viral gene expression exhibits a sequential and regulated pattern that is influenced at both the transcriptional and post-transcriptional levels.⁶

CMV gene expression and regulation

In general, CMV gene expression may be divided into three sequentially expressed kinetic classes: α (immediate early), β_1 and β_2 (delayed early), and γ_1 and γ_2 (late) based on the time of expression after virus infection.^{3,7} Four classes of α gene expression have been mapped on the human CMV genome, UL36-38, *ie1/ie2*, TRS1-IRS1, and US3. The expression of α genes does not require *de novo* protein synthesis and the majority of α gene products are synthesized from a single IE locus between the 169 and 175 kb pairs on the human CMV genome immediately after virus infection. The *ie1* and *ie2* gene products have been implicated in both positive and negative regulation of viral gene expression. Expression of β and γ gene products requires the synthesis of functional α gene products which act as transcriptional *trans*-activators.

Delayed early β_1 genes are transcribed between 4 and 8 hours post infection while β_2 genes are expressed at around 8 and 24 hours postinfection. Synthesis of β genes requires new proteins and the messages from both of these gene types appear before the onset of viral DNA synthesis. Most of these genes products, such as viral DNA polymerase, are involved in viral DNA synthesis.

Virus DNA Polymerase

HCMV DNA polymerase (pol) is the central enzyme in viral DNA replication and as a result, it has become an easy target for many antiherpetic agents like the triphosphates of nucleoside analogs (such as ganciclovir triphosphate) and the pyrophosphate (PP_i) analog such as foscarnet. It is a 140 kD protein, consisting of 1242 amino acids (HCMV strain AD169) and its range of activities include a 3' to 5' exonuclease proof-reading activity to increase fidelity. The HCMV DNA polymerase gene (UL54) along with several other β -gene products are likely to be involved in viral DNA synthesis.^{8,9}

Both DNA and amino acid sequence analyses have revealed that the gene coding for HCMV pol has significant homology to the pol genes of other herpesviruses such as herpes simplex virus type 1 (HSV-1) and Epstein-Barr virus (EBV).¹⁰ In addition, it is now known that herpesvirus DNA polymerases share striking sequence similarities with eukaryotic DNA polymerases α and δ and many other DNA polymerases of viruses and bacteriophages in several discrete regions that define a family of α -like polymerases.^{11,12} There are eight regions in the same spatial order in various herpesvirus family members and have been designated I through VII and A, in decreasing order of similarity. All eight conserved regions exhibit similar linear spatial arrangements in the order of IV-A-II-VI-III-I-VII-V although the relative distance of each of these regions in various polymerases may vary from each other.¹³

Despite these similarities, HCMV pol, like HSV-1 pol, has been distinguished from the cellular pol α by its chromatographic behavior, template-primer specificity, requirement for higher salt concentrations for maximum activity and sensitivity to inhibitors such as the triphosphates of famciclovir and acyclovir. Sequence analysis of HSV-1 and CMV pol mutants have demonstrated that many antiviral drugs mimic and/or compete with deoxynucleoside triphosphate (dNTP) or PP_i at specific sites within regions I, II, III, V, VII and A. Furthermore, some of these regions may interact with each other in a way that allows viral pol to be more sensitive to various antiviral drugs than human DNA pol α .¹⁴ Site-directed mutagenesis analysis of the polymerase has allowed the association of conserved regions with particular enzyme functions. For example, conserved region III (amino acid residues 804 to 845) appears to be involved with DNA template binding, and residues between regions II and V (amino acid residues 725 to 964) are involved in deoxyribonucleoside triphosphate binding.¹⁵

A protracted early phase of gene expression, lasting more than 24 hours is characteristic of CMV infection and is a major contributing factor to its slow replicative cycle. Late γ_1 transcripts are detected between 12 and 36 hour postinfection coinciding with active viral DNA synthesis. Kinetic studies have shown that the HCMV UL97 gene product, a protein kinase homologue (see below), is in the γ_1 class of viral proteins based on the time of its expression. Maximal expression of UL97 requires viral DNA synthesis. The γ_2 transcripts of HCMV are detected between 24 and 48 hours postinfection. Structural components of the capsid begin to accumulate, other viral components assemble and newly synthesized virions are released (by budding) through the plasma membrane.

HCMV protein kinase, UL97

The open reading frame (ORF) UL97 of human CMV DNA encodes a protein that has homologies with protein kinases, directs phosphorylation of GCV to GCV monophosphate,^{16,17} and may be essential for CMV growth.¹⁸ Experiments with recombinant vaccinia viruses containing a range of mutant UL97 genes have suggested that the N-terminal region contains a nuclear localization signal. The C-terminus may be involved in binding and phosphorylating GCV and the central portion may have a role in phosphorylating both GCV and UL97 protein (pUL97) itself.¹⁹

The UL97 gene has conserved homologues in all herpes sub-families.¹⁸ Indeed, the UL97 gene product has been found to substitute in part the functions of the HSV-1 UL13 gene product. Autophosphorylative protein kinase activity of the UL97 gene product has been demonstrated for both recombinant protein prepared from insect cells and viral-expressed UL97 immunoprecipitated from virions and infected cells. Like other protein kinases from different species, several domains, I to XI, have been defined by sequence alignments. In pUL97, homologous sequences have been found for the protein kinase domain.^{20, 21}

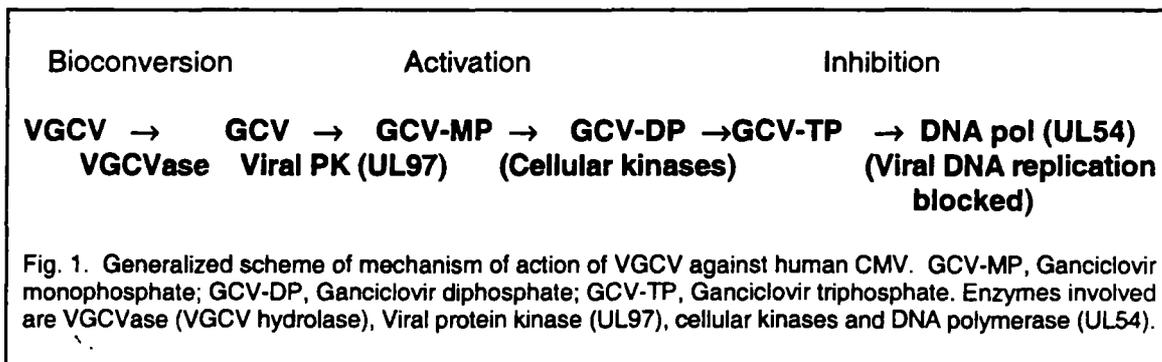
CMV Infection

In productive infection, the entire replicative cycle of CMV in a suitable susceptible host is approximately 72 hrs. However, infection of the normal host with HCMV is followed by a complex series of both humoral and cellular immune responses against the virus. These include production of IgG and IgM antibodies, responses to cytotoxic T cells, and activation of natural killer (NK) cells and antibody-dependent killer cells. HCMV specific antibodies with neutralizing as well as complement fixing activities are produced and such antibody responses may serve to limit the replicative capacity of the virus. Along with the HCMV-specific immunoglobulins, various aspects of cell-mediated immune responses due to HCMV infection may keep the virus in a latent form for an extended period of time. Reactivation of the virus usually occurs when the immune surveillance system in such hosts is further suppressed either by other viruses such as HIV or by pharmacologic manipulations needed for organ transplant patients.

Many cells of nonhuman origin can undergo abortive infection with CMV. Various types of cytopathology associated with the synthesis of viral antigens are characteristics of abortive infection. Usually a limited number of viral components are synthesized and these components do not assemble to form a complete virion. Inoculation of cells, both of human and nonhuman origin, with infectious or UV inactivated CMV may lead to oncogenic transformation. Such transformed cells are capable of producing malignant tumors in indicator animal models.

A. MECHANISM OF ACTION OF VGCV

As indicated earlier, VGCV must be converted initially to GCV, which in turn is metabolized to GCV triphosphate (GCV-TP) to inhibit viral DNA synthesis. A general scheme of the metabolic pathway and the enzymology involved in the conversion of VGCV to GCV-TP to finally inhibit viral DNA replication is presented in Fig. 1 below.



A.1. Conversion VGCV to GCV

The sponsor has stated that after oral administration, VGCV is rapidly hydrolyzed to GCV and L-valine by intestinal and hepatic esterases. *In vitro* studies using human and animal (dog and mouse) intestinal and hepatic fractions have shown that VGCV is metabolized to GCV with no other metabolites having been identified. The sponsor has further stated that the proposed once daily dosing with VGCV can achieve plasma exposures of GCV comparable with standard regimens of intravenous GCV. In this regard, please refer to the pharmacologist's review for details.

A.2. Activation of GCV by phosphorylation

GCV is essentially a prodrug without any intrinsic antiviral activity; it must be phosphorylated to an antivirally active triphosphate form in virus-infected cells to inhibit viral DNA replication thus exerting its antiviral activity. In CMV-infected cells, GCV is anabolised to its triphosphate form primarily by 3 enzymes:

1. The CMV encoded protein kinase, UL97, carries out the critical initial phosphorylation step to produce GCV-MP.^{16,17,22} Mutations in UL97 may reduce the capacity of infected cells to phosphorylate GCV. In addition, the inclusion of UL97 in a recombinant vaccinia virus confers the ability of infected cells to phosphorylate GCV. It should be noted that in contrast to HSV, which encodes a viral thymidine kinase (TK) to carry out the first phosphorylation of GCV, CMV does not appear to encode a TK gene. On the other hand, the homolog of UL97 in HSV-1, which is also a protein kinase, is not involved in the initial phosphorylation of GCV in HSV-1 infected cells.

2. Cellular guanylate kinase phosphorylates the monophosphate to GCV diphosphate.²³

3. Cellular phospho-glycerate kinase is the principle enzyme to carry out the last phosphorylation to produce the active moiety, GCV-TP²³. Once phosphorylated, the final antivirally active GCV-TP appears to persist with a half-life >6 to <24 hours in HCMV infected cells.²⁴

A.3. Inhibition of Viral and Cellular DNA Polymerases

GCV-TP is both inhibitory to and a substrate for the viral DNA polymerase, thus preferentially (but not exclusively) inhibiting viral DNA synthesis. Experiments have also demonstrated that GCV-TP functions as a competitive inhibitor of the incorporation of deoxyguanosine triphosphate (dGTP) into HCMV DNA. The K_i for GCV-TP against HCMV DNA polymerase from different strains has been estimated in the range 0.022 to 1.7 μM (Table 1 below). GCV-TP also acts as an inhibitor of cellular DNA polymerase α , but to a lesser extent than HCMV viral DNA polymerase, with K_i range of 0.13 – 24 μM . Activity against cellular DNA polymerase β would appear to be slight (Table 1).

GCV-TP is also known to be incorporated into DNA. However, it does not act as an obligate chain terminator. Incorporation and elongation may occur by virtue of the presence of hydroxyl groups analogous to the natural substrate's 3'- and 5'- hydroxides. DNA with GCV-MP at its 3'-terminal provides a poor substrate for continued chain elongation and results in a reduced rate of DNA synthesis. Incorporation of GCV has been reported to change the physico-chemical properties of DNA, and the linkage has been found to be alkali-labile.

B. ANTIVIRAL ACTIVITY

B.1. Single Drug Studies *In Vitro* :

Ganciclovir is a potent inhibitor of a number of herpesviruses pathogenic to both man and animals. Its potency to inhibit various laboratory strains or clinical isolates has been reported to be dependent upon a number of factors including the host cells, virus strain, multiplicity of infection, assay method,

Table 1. Activity and specificity of GCV-TP as substrates of DNA polymerases

DNA polymerase	Km (dGTP) (μM)	Ki (GCV-TP) (μM)	Reference ¹
HCMV (AD169)	N.E.	1.4 \pm 0.27	[7418]
HCMV (Towne)	0.47	0.022	[7519]
HCMV (AD169)	0.57	1.7	[7457, 7476]
HSV-1 (KOS)	0.24	0.033	[7470]
HSV-1 (KOS)	0.17 \pm 0.01	0.05 \pm 0.01	[7576]
HSV-1 (Patton)	0.18 \pm 0.01	0.08 \pm 0.01	
HSV-1 (PAA ^R)	0.68 \pm 0.006	0.05 \pm 0.01	
HSV-1 (BW ^R)	0.61 \pm 0.19	0.05 \pm 0.01	
HSV-1 (F)	N.E.	0.09	[7561]
HSV-1 (Patton)	0.96 \pm 0.35	0.31 \pm 0.05	[7478]
HSV-2	0.12	0.046	[7470]
MRC-5 (α)	2.2	17	[7457, 7476]
Peripheral blasts (AML patient) (α)	0.8	0.13	[7470]
WI-38 (α) ^o	1.4	0.15	[7519]
HeLa S-3 (α) ^p	1.2 \pm 0.22	2.5 \pm 0.7	[7418, 7576]
HeLa (α) ^p	N.E.	4.2	[7561]
HeLa (α)	1.1 \pm 0.50	24 \pm 13	[7478]
	0.42 \pm 0.15		
HeLa S3 (β) ^p	N.E.	Not measurable*	[7547]
HeLa (β)	0.33	338 \pm 235	[7478]

^o Human fibroblasts

^p Human cervical carcinoma cells

Kinetic constants (Km for dGTP and Ki for GCV-TP) are shown for HSV-1, HSV-2 and HCMV viral and cellular DNA polymerases (α and β) with published references. NE, Not estimated.

¹References in the Table are described in Vol. 83 of this NDA.

and even the laboratory personnel. Thus, the concentration of GCV that inhibits the replication of various human herpesviruses by 50 % (IC₅₀) have ranged significantly as follows: Human CMV, 0.01-27 μM ; HSV-1, 0.2-8.0 μM ; HSV-2, 0.1-11.8 μM ; EBV, 0.05-125 μM ; and VZV by 3.3-51 μM . Summary of antiviral activity of GCV and acyclovir (ACV) against different herpesviruses as measured by different assay procedures is presented in Appendix 2, Vol. 87 of this NDA submission. The IC₅₀ values of different laboratory strains and clinical isolates of human CMV, relevant to the review of NDA are presented in Table 2 below. The sponsor has stated that one determination, in which the endpoint was obtained by measuring the reduction of fluorescent foci, failed to detect any anti-CMV antiviral activity of GCV at a concentration of 100 $\mu\text{g/mL}$ (400 μM).

inhibited cells. *In vitro* studies of the proliferation of bone marrow cells have also shown a greater sensitivity of granulocyte-macrophage colony forming units (CFU-GM) ($TD_{50} = 2.7 - 12 \mu M$) and of erythroid burst-forming units (BFU-E) ($TD_{50} = 4.6 \mu M$) to GCV than that observed with most common tissue culture cells. Thus, while the therapeutic index of GCV may be in the acceptable range in some of the host cell lines tested, it may be quite low in rapidly dividing cells (e.g., hematopoietic progenitor cells) and may prove to be highly toxic.

Table 4. Cytotoxicity of GCV and ACV (when available) in different cell lines using different endpoints

Reference ¹	Cell line	Assay (endpoint)	GCV cytotoxicity	ACV cytotoxicity
[7571]	HEL	Cell growth inhibition (TD_{50})	>100 μM	-
[7509]	P3HR1, Raji	Cell growth (TD_{50})	200 μM	250 μM
[7603]	Guinea pig embryonic fibroblasts	Cell proliferation	>260 $\mu g/mL$	N.E.
[7605]	GPE cells	Viable cell count (TD_{50})	746 μM	656 μM
[7531]	BCBL-1 (HHV-8)	Cell growth	354±171 μM	≥690 μM
[7507]	Vero	Cell proliferation (TD_{50})	270 μM	-
	MRC-5	CFU estimation (TD_{50})	>500 μM	-
	Bone marrow CFU-GM	CFU estimation (TD_{50})	12 μM	
	BFU-E	BFU-E estimation (TD_{50})	4.6 μM	
[7564]	Vero	Cell proliferation (TD_{50})	850 μM	850 μM
[7562]	Vero	Cell proliferation (TD_{50})	900 μM	1400 μM
[7573]	CFU-GM	CFU inhibition (TD_{50})	2.7±0.5 μM	>100 μM
[7457]	MRC-5	Cell proliferation (TD_{50})	>1000 μM	N.E.
[7543]	Brain cell aggregate (primary culture)	Histology	>200 $\mu g/mL$	>200 $\mu g/mL$
[7418]	MRC-5	Cell proliferation (TD_{50})	350 μM	3000 μM
[7476]	MRC-5	Cell proliferation (TD_{50})	110±50 μM	1575±140 μM
	HET		250±80 μM	1360±260 μM
	SML		1500±950 μM	1820±980 μM
	GPE		2900±84 μM	1200±650 μM
	MEF		210±80 μM	180±70 μM
[7426]	HeLa-S3	Cell growth (TD_{50})	125	-
	WI-38		>50 μM	-
	P3HR-1		40 μM	-
[7568]	HFF-86-P-5	Cell proliferation (TD_{50} , $\mu g/mL$)	10 [0.01]	-
	HFF-124-P-7		60 [0.021]	-
	HFF-118-P-3	[HSV-1 Patton ED_{50}]	70 [0.16]	-
	HFF-127-P-6		43 [0.3]	-
	Vero		135 [0.31]	-
	HFF-36-P-6		32 [0.09]	-
[7576]	Vero	Cell proliferation (TD_{50})	460 μM	300 μM

¹References cited in this table are described in Vol. 83 of this NDA.

B.2. Drug Combination Studies *In Vitro* :

When GCV is combined with other anti-CMV compounds, anti-HIV drugs and drugs used commonly against other AIDS-associated opportunistic infections, additive or synergistic activity against HCMV has been observed. GCV may antagonize the anti-HIV activity of zidovudine (AZT) and didanosine (ddI). On the other hand, the immunosuppressant, mycophenolate mofetil MMF, may potentiate GCV antiherpes activity. Cytotoxic interactions have been found to be additive, although synergistic cytotoxicity may occur with the GCV/AZT combination.

Two methods have been employed to examine these antiviral interactions. In one method, one drug is titrated in the presence or absence of a constant concentration of the other. The endpoints for each virus are set as equivalent to 1.0 'fraction of inhibitory concentration' (FIC). The FIC for each drug can then be estimated in each titration and a plot of the FIC of one drug against that for the other can be drawn. The sum of FIC (combination index, CI) then gives an indication of the type of interaction. Additive interactions provide points along the diagonal line between the two points for the drugs alone (i.e. CI = 1.0). Data points above this line indicate antagonism (CI > 1.0) and points below the line indicate synergy (CI < 1.0). One convention sets limits for synergy if the CI is ≤ 0.5 and for antagonism if the CI is ≥ 1.5 .

In the second method, a checkerboard assay of one drug versus the other drug at varying concentrations is carried out. The data is subject to a 'universal response surface approach', which allows the calculation of a synergism-antagonism parameter (α). This method has the advantage of designating antagonistic or synergistic interactions on statistical grounds. Results of some of the studies related to this NDA are summarized as follows.

B.2a. Ganciclovir and Foscarnet on CMV

GCV alone inhibited the plaque formation of human CMV (strain T86180) in human embryonic lung fibroblasts (MRC-5) with an IC_{50} value of 9 to 10 μM , while foscarnet was moderately active with an IC_{50} value of, 116 to 134 μM . Various combinations of GCV (5-30 μM) and foscarnet (25-800 μM) demonstrated an additive effect on plaque reduction. Combination of these two compounds also demonstrated an additive cytotoxic effect on proliferating MRC-5 cells. Additive anti-HCMV antiviral and cytotoxic interactions have been reported between GCV and foscarnet (PFA) in MRC-5 cells infected with a laboratory strain of HCMV (AD169) or with a clinical isolate.

B. 2b. Ganciclovir and Zidovudine on HCMV

Zidovudine was relatively inactive to inhibit plaque formation by CMV (AD169 strain) in MRC-5 cells with an IC_{50} value of 508 to >800 μM while GCV alone was much more active with an IC_{50} value of 8 to 9 μM . Various combinations of GCV (5-30 μM) and zidovudine (25-800 μM) demonstrated a range of anti-CMV antiviral interactions between GCV and zidovudine have been reported (Table 5). No interaction (or an effect within 2-fold of that observed with GCV alone) has been reported in some studies (i.e. zidovudine did not significantly enhance or reduce the antiviral effect of GCV). However, up to 9-fold potentiation of GCV antiviral activity has been observed with zidovudine. Potentiation of antiviral effect was also observed in the same system with clinical isolates. Modest reduction (2-fold) of GCV anti-HCMV antiviral activity has been observed with AZT in one study.

Synergistic cytotoxicity of GCV with zidovudine has been reported in HEL cells, although the effect was < 2-fold. In KB (human epidermoid carcinoma), HFF and WI-38 (human embryonic diploid) cells, synergistic cytotoxicity has also been observed between GCV and zidovudine using cell proliferation and plating efficiency assays. Tests in the H9 T-lymphoblastoid cell line have also

indicated some tendency for synergistic cytotoxicity between GCV and zidovudine, but found additive cytotoxicity between GCV and ddl.

Table 5. Anti-HCMV antiviral interactions between GCV and Zidovudine

Virus/cell line	Assay/ Endpoint	Interaction	Reference ¹
HCMV (AD169)/MRC-5	pfu; IC ₁₀₀ *	Potentialiation	[7619, 7638]
HCMV (AD169)/MRC-5	pfu; IC ₅₀ /IC ₉₀	No interaction	[7630]
HCMV (clinical isolate)/ MRC-5	pfu; IC ₅₀	Potentialiation	[7635]
HCMV (lab and clinical strains)/HEL	pfu; IC ₅₀	Potentialiation	[7571]
HCMV (AD169)/HEL	pfu; IC ₅₀	Reduction	[7467]

*Lowest concentration at which all plaque formation was inhibited.

¹Cited references are described in Vol.83 of the NDA.

B.2c. GCV and Zidovudine on Human Immunodeficiency Virus (HIV)

The effect of zidovudine (0.00006 - 1.0 µM)-or GCV (0.005 - 30 µM) alone or in combination against HIV-1 (LAV strain) was tested in a human lymphoblastic T cell line (A3.01). Zidovudine alone inhibited HIV-1 with an IC₅₀ value of 0.001-0.014 µM, while GCV alone was ineffective with an IC₅₀ >30 µM. When combined together, GCV did not exert any effect on the anti-HIV activity of zidovudine. GCV has been found to be inactive at concentrations >30 µM against HIV-1 in A3.01 cells infected with HIV-1_{LAV}. In an analysis of GCV combined with zidovudine, an antiviral interaction was not reported. However, a 3- to 6-fold reduction of anti-HIV potency of AZT and ddl in combination with GCV has been reported in H9 cells infected with HIV_{HTLV-IIIB}.²⁵

B.2d. Antiviral and cytotoxic activity of GCV in combination with other agents

The sponsor has stated that anti-CMV antiviral interaction has not been observed between GCV and amphotericin B, dapsone, ketoconazole, trimethoprim/sulphamethoxazole, mycophenolic acid and trequesin.

B.3. *In Vivo* Studies with Single Drug GCV:

Because of strict species specificity, human CMV does not have an appropriate animal model. Therefore, anti-CMV activity of GCV in a murine model with murine cytomegalovirus (MCMV) or in a guinea pig model with guinea pig cytomegalovirus (GPCMV) has been extensively studied. However, it should be pointed out that it is difficult to compare results from different *in vivo* studies. Even within studies of the same animal model, virus strain, inoculum and route of infection, compound dosage, route of administration and time between infection and the first dose might differ. In addition, the duration of dosing, the follow-up period and the chosen therapeutic endpoint might limit the comparative results. As a result, the clinical relevance of animal models in CMV infection is yet to be established.

B.3a. MCMV in Mouse Model

The sponsor has described an experiment where weanling mice infected intraperitoneally (i.p.) with a lethal dose (3.2 x 10⁴ PFU) of MCMV (Smith) and treated with GCV subcutaneously (s.c.) (≥10 mg/kg/dy b.i.d.) for 5 days, starting 6 hours postinfection. Results presented in Table 6 (see below) shows that there was a significant increase in survival time of the mice administered with the drug.

Of the mice that died, a significant increase in the mean survival time was observed at the minimum dose (1.0 mg/kg/dy s.c.)²⁶ (Table 6). Delayed dosing at 50 mg/kg/dy s.c. also gave rise to a significant reduction in mortality when therapy was started 48 h post-infection (Table 6).

Table 6. Antiviral activity of GCV against MCMV *in vivo*

The survival rate and the mean survival time of dying mice is shown for treatment with different doses of GCV (experiment 1) and with 50 mg/kg/day s.c. GCV, starting at different times postinfection (experiment 2).(adapted from reference 7478¹)

Dose (mg/kg/dy, s.c., b.i.d.)	Time started (h post-infection)	Surviving mice/ total mice	Mean survival time of dying mice (days±SD)
0	6	2/20	4.4±0.78
1	6	2/20	6.2±1.8*
5	6	2/20	6.3±1.4*
10	6	8/20*	7.7±1.8*
25	6	15/20*	6.4±0.6*
50	6	19/20*	7.0*
0	6	2/19	5.2±1.2
50	6	18/20*	6.5±0.71
50	24	15/19*	11±3.8*
50	48	9/19*	8.0±2.5*
50	72	6/20	6.1±1.6
50	96	1/20	4.8±0.71

*Statistically significant difference from control group, which received saline only from 6 h post-infection.

¹Cited reference 7476 is described in Vol. 83 of this NDA.

The sponsor has also stated that protection of immunosuppressed mice has also been assessed in severe, combined immunodeficient (SCID) mice, which are highly susceptible to murine MCMV infection. SCID mice infected i.p. with $10^{3.5-4.5}$ PFU MCMV and dosed b.i.d. with 25 and 50 mg/kg GCV from 24 h postinfection for 10 days., resulted in delay of death by 2 to 8 days.²⁷ Treatment with GCV at 75mg/kg was not found to be toxic, however, mice did not survive therapy with 150 mg/kg doses.

B.3b. HCMV in SCID Mice

In vivo anti-HCMV antiviral activity of GCV has been demonstrated in the SCID-hu-mouse model. Co-implants of human fetal thymus and liver infected with human CMV (Toledo strain) supported high level CMV replication up to 9 months in SCID-hu-mice²⁸. Only the human tissue implants, but not the surrounding mouse tissues, supported human CMV replication. GCV administered in drinking water (0.125 to 1.5 mg/ml) 6 hrs after inoculation or intraperitoneally at 8 or 10 mg/kg/day reduced the virus titers in tissue samples of the order of $10^2 - 10^4$ fold.

B.3c. GPCMV in Guinea pig Model

Guinea pig CMV (GPCMV) is relatively insensitive to GCV *in vitro* (IC_{50} 36 – 70 μ M) and represents a challenge for *in vivo* study. In one study, guinea pigs inoculated i.p. with GPCMV (strain 22122), were treated from 24 h postinfection with 25 mg/kg b.i.d. s.c. for 7 days. Virus infection resolved naturally after antibody detection at about 13 days p.i. in blood, spleen and lung of treated and untreated guinea pigs. However, virus persisted in salivary glands at significantly lower titers in

treated than untreated animals. Lesion scores did not differ in spleens, livers and lungs, but were significantly less severe in kidneys and salivary glands of treated animals after 21–28 days.²⁹

B.4. *In Vivo* Studies of GCV Combined with Other Drugs

To determine whether combination of GCV with other commonly used antiviral agents (especially against HIV and HIV-associated opportunistic infections) such as foscarnet (PFA) or zidovudine (ZDV) or antimicrobial agents such as amphotericin B or ketoconazole will influence the anti-CMV activities of GCV a murine CMV (MCMV) model was used. Results summarized in Table 7 show that the anti-MCMV activity of GCV was not affected by any of the compounds listed in the table. Apart from GCV, only PFA showed any protection against CMV infection. The interaction between GCV and PFA was additive. However, the significance of these results to human CMV in a clinical setting can not be evaluated.

Table 7. The *in vivo* efficacy of GCV and other drugs alone or in combination against MCMV infection of mice

The doses required for individual drugs to give rise to 50% survival of mice in the presence or absence of a given concentration of a second drug are shown.

Study References ¹	Compound(s)	ED ₅₀ alone or in combination (mg/kg/dy)
[7617]	GCV (s.c., b.i.d.) AZT (i.p., t.i.d.) Varying GCV + AZT (320 mg/kg/dy) Varying AZT + GCV (3 mg/kg/dy)	8 >320 7 >320
[7624]	GCV (s.c., b.i.d.) PFA (i.p., t.i.d.) Varying GCV + PFA (300 mg/kg/dy) Varying PFA + GCV (3 mg/kg/dy)	3 ≥300 <1 <96
[7614]	GCV (s.c., b.i.d.) Amphotericin B (p.o., qd) Varying GCV + Amphotericin B (20 mg/kg/dy) Varying Amphotericin B + GCV (3 mg/kg/dy)	17 >20 18 >20
[7614]	GCV (s.c., b.i.d.) Ketoconazole (i.p., qd) Varying GCV + Ketoconazole (60 mg/kg/dy) Varying ketoconazole + GCV (3 mg/kg/dy)	5 >60 4 >60
[7615]	GCV (s.c., b.i.d.) Dapsone (po, b.i.d.) Varying GCV + Dapsone (32 mg/kg/dy) Varying Amphotericin B + GCV (3 mg/kg/dy)	12 >32 10 >32
[7615]	GCV (s.c., b.i.d.) Trimethoprim/Sulfamethoxazole (TMP/SMX) (po, b.i.d.) Varying GCV + TMP/SMX (80/400 mg/kg/dy) Varying TMP/SMX + GCV (3 mg/kg/dy)	9 >80/400 9 >80/400
[7611]	GCV (s.c., b.i.d.) Papaverine (s.c., b.i.d.) Varying GCV + Papaverine (60 mg/kg/dy) Varying Papaverine + GCV (3 mg/kg/dy)	3 >60 5 >60

¹References are described in Vol. 83 of the NDA.

C. VIRAL RESISTANCE

It is now well recognized that HCMV resistance to GCV results from mutations in its UL97, UL54 or both of these genes. Mutants of HCMV resistant to GCV have been isolated from cultured cells *in vitro* as well as from immunocompromised patients undergoing treatment for HCMV infection. Serial

passage of HCMV in the presence of increasing concentrations of GCV has resulted in the emergence of mutants exhibiting IC₅₀ values of 10 - fold greater than that of the wild type strain. However, the degree of HCMV resistance to GCV is not always clearly distinguishable within the normal range of wild-type CMV sensitivity. Analysis of several wild-type and resistant viruses may establish "cut-off" IC₅₀ values or fold-increases of IC₅₀ that represent significant changes from the wild-type range of IC₅₀ values for any one assay. These definitions are determined from assay sensitivity and variability. There are currently a number of different assays and endpoints used to determine the susceptibility of HCMV to inhibition with GCV. Susceptibility testing, which is yet to be standardized, has been carried out most often by plaque reduction assay using adapted or recombinant laboratory viruses and clinical isolates. The current working definition of CMV resistance to GCV is when GCV IC₅₀ ≥ 1.5 µg/ml (≥ 6.0 µM).

C.1. Generation of HCMV resistance to GCV *In vitro*

As noted earlier, a protein kinase homolog encoded by the CMV UL97 open reading frame and CMV DNA polymerase (encoded by the CMV UL54 reading frame) are two key enzymes recognized thus far to be responsible for the anti-CMV activity by GCV. Therefore, resistance to GCV has been generated by mutations in these two viral genes; the gene encoding the kinase which monophosphorylates GCV and the viral DNA pol which is the ultimate target of GCV-TP. The first proof that a four amino acid (Ala-Ala-Cys-Arg, residues 638-641) deletion of UL97 in a conserved region (which in cyclic AMP-dependent protein kinase participates in substrate recognition), caused impaired GCV phosphorylation in HCMV-infected cells, was provided by Sullivan and coworkers in 1992.¹⁷

Generation of CMV mutants resistant to GCV was achieved in studies of *in vitro* passage of virus in the presence of increasing levels of GCV.³⁰ A 12-fold reduction in sensitivity to GCV was obtained and the defect was traced to mutations in both UL97 and UL54. Reduced capacity for the virus-infected cells to phosphorylate GCV was observed. A 12 base pair deletion (Δ) in UL97 (Δ 591 – 594) was found, and shown in a recombinant virus to contribute to a 3- to 10- fold reduction in sensitivity to GCV.¹⁷ In a more recent study, it has been demonstrated that mutations at amino acids G340, A442, L446, and F523 resulted in a complete loss of pUL97 phosphorylation, which was strictly associated with GCV phosphorylation.²¹ In this study, the mutation in UL54 was also characterized (A987G) and shown to contribute to resistance.¹²

C.2. Characterization of virus resistant to GCV in clinical isolates

Several studies have been carried out to characterize resistant clinical isolates of HCMV and *in vitro*-derived viruses. A listing of mutations and their effects on phenotypic sensitivity to GCV and other anti-HCMV antiviral drugs relative to wild-type virus (fold IC₅₀ change) is provided in Appendix 4, Vol. 83 of this NDA. Resistance of clinical isolates to GCV has been estimated to occur primarily through the mutation of UL97 in about 90% of instances and UL54 in the remaining 10%.³¹ The contribution of many of the mutations observed in clinical isolates has been assessed in recombinant viruses.

C.2a. Susceptibility Tests

1. The classic plaque reduction assay is still popular and routinely used to assay virus susceptibility to drugs even though the procedure itself is yet to be standardized. This procedure also suffers from technical difficulty to culture primary clinical isolates needing to be passaged several times (typically 5–6), which in itself is a very time consuming procedure. As this might result in the loss of less fit mutant virus species, methodologies that do not require extensive passage of virus prior to testing

have been developed. For example, the shell vial assay provides rapid drug susceptibility data by the more sensitive detection of late viral antigen.³² This assay can be carried out more rapidly and with less chance of loss of mutations.

2. More recently, antiviral susceptibility testing by flow cytometric analysis (AST-FCA) was developed.³³ This methodology appears to give good correlation with plaque reduction assay results and provides high inter-laboratory reproducibility. A series of GCV-sensitive isolates yielded a mean IC_{50} of $3.8 \pm 2.6 \mu M$ compared with $2.8 \pm 1.5 \mu M$ by plaque reduction. In addition, the use of antibody may provide increased specificity as adenovirus plaques may contaminate plaque reduction assays in clinical specimens.

3. Results of phenotypic assays may vary between laboratories. Viral genotype may be obtained directly from patient PBMC through PCR amplification of viral DNA, which is more reliable and faster than virus isolation. The viral DNA may be evaluated either by restriction digest analysis³⁴ or sequence determination of relevant regions of the viral genome.^{35,36} Interpretation of genotype may be carried out using known relationships between certain mutations and drug susceptibility. These relationships have been established by the characterization of mutant viruses that were selected during *in vitro* passage in the presence of the drug, or during patient treatment with the drug, and by studies of recombinant viruses with known single or multiple resistance mutations. Because of the speed of determination, the genotype may be used to help make informed therapeutic choices.

C.2b. Resistance mutations in UL97 from clinical isolates

As discussed earlier, mutation of the UL97 gene presents the most common mechanism for virus to derive resistance to GCV *in vivo*.³¹ The sponsor has compiled the results of various studies demonstrating the GCV mutational hot spots (1 and 2) in the various domains of UL97 (Fig.2). Mutations in the putative ATP-binding site (M460V), GCV binding site (residues 590 – 607), and the proposed catalytic domain (L643S) of UL97 in GCV-resistant clinical isolates have been observed. Reduced capacity of recombinant virus with the H520Q substitution to phosphorylate GCV has also been demonstrated.³⁷ Studies with UL97-recombinant vaccinia virus confirm impaired phosphorylation of GCV with the mutations M460V, H520Q, $\Delta 590-593$ or A594V.¹⁹

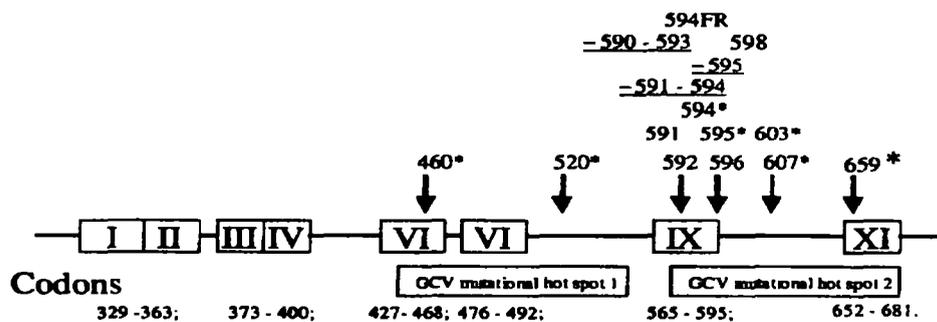


Fig. 2. Map of amino acid substitutions in UL97 associated with GCV resistance
Amino acid substitutions in human CMV UL97 associated with reduced sensitivity to GCV are shown relative to conserved regions of the protein. Single amino acid substitutions, frame shift mutations or deletions are shown. Substitutions whose role has been confirmed by marker transfer are denoted *, -, and underlines, denote deletions and FR denotes a frame shift. (Adapted from Fig. 5, page 68, Vol. 83 of this submission. Conserved region VI under 460* arrow should be *Via* and the next conserved region VI should be labeled as *Vib*).

C.2c. Resistance mutations in UL54 from clinical isolates

As indicated earlier, resistance to GCV may occur due to mutations in UL54, although relatively infrequently. Analysis of the UL54 gene has revealed substitutions in the DNA sequence of its conserved regions arising during treatment with GCV and these substitutions have been shown to be associated with GCV-resistance.³⁸ A map of the amino acid substitutions in various regions of UL 54 associated with GCV resistance as presented by the sponsor is reproduced in Fig. 3 below.

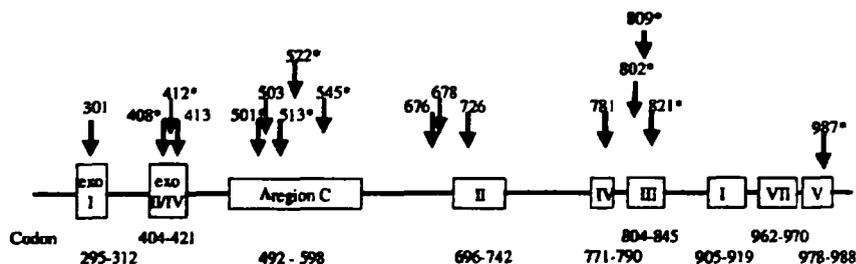


Fig.3. Map of amino acid substitutions in UL54 associated with GCV resistance
Amino acid substitutions in human CMV UL54 associated with reduced sensitivity to GCV are shown relative to functional regions of the protein. Substitutions whose role has been confirmed by marker transfer experiments are denoted *. (Adapted from Fig. 6, page 69, Vol. 83 of this submission).

Attempts have been made to rationalize patterns of resistance and cross-resistance by delineating regions of UL54 where different drugs might be affected by amino acid substitutions (Table 8). Region II (T700A and V715M) and region VI (V781I) mutations have been associated with resistance to PFA and adefovir (ADV). Region II may also affect sensitivity to lobucavir (LBV). Region III mutations (L802M, K805Q, T821I) showed a variety of phenotypic resistance patterns.³⁹

Table 8. IC₅₀ changes due to UL54 single point mutations¹

Relative fold-change of IC₅₀ of recombinant HCMV expressing single point mutations in the UL54 gene compared with wild type virus are shown for GCV, cidofovir (CDV), PFA, ADV and LBV.³⁹

Region	UL54 substitution	Fold change in IC ₅₀ relative to wild-type (resistant only shown)				
		GCV	CDV	PFA	ADV	LBV
IV	N408D	4.9 ^a	5.6	1.3	-4.0	1.6
	F412C	4.2	18	1.2	-4.2	1.2
	F412V*	4.3	16	1.1	-4.2	1.0
δ C	L501I*	6.0	9.1	1.4	-2.0	1.4
	K513E	5.0	9.1	1.4	-2.0	1.2
	P522S	3.1	3.6	1.1	-2.5	1.1
	L545S	3.5	9.1	1.2	-5.9	1.2
	D588E	1.3	1.1	2.3	2.0	1.1
II	T700A	1.2	1.3	5.8	5.8	3.1
	V715M	1.3	1.1	9.5	6.0	3.1
VI	V781I	1.0	1.2	5.2	3.0	1.8
III	L802M	1.1	0.9	3.2	2.8	1.3
	K805Q	1.0	2.2	-5.6	-4.8	-4.1
	T821I	4.5	1.9	21	6.4	3.6
V	A987G*	5.3	11	1.2	1.1	1.1
Nonconserved region	S676G	1.1	1.2	0.9	1.4	1.0
	V759M	1.5	1.1	1.1	1.2	1.3

^a Boldface numbers represent significant changes in HCMV drug sensitivity. Negative values represent hypersensitivity

*Observed during *in vitro* selection.

¹ From Table 11 (page 70, Vol. 83)

Comment: It should be pointed out that the physical map of the conserved regions of the UL54 gene presented in Fig.3 above is different than the maps generally published by others.^{14,15,40}

By analyzing the HCMV DNA pol gene coding sequences of 40 independent clinical isolates, Chou et al.(1999)⁴⁰ have recommended that genotypic assays for resistance should focus on the *pol* codon ranges 379 to 421, 492 to 525, 696 to 845, and 978 to 988.

C.2d. Cross-resistance patterns of mutant HCMV generated in the clinic

Recent data from clinical studies show that cross-resistance to other anti-HCMV antiviral agents is observed in a minority of patients who have developed resistance to GCV. Two mutations in UL54 have been associated with cross-resistance between GCV and PFA. These are D301N (DNA polymerase conserved region exo I) and A809V.⁴¹ Treatment with both GCV and PFA may lead either to true cross-resistance or to “dual resistance” as a consequence of selective pressure from both drugs selecting for their respective resistant mutants.

C.2e. Modulation of viral properties

Recent data show that the ability of resistant virus to replicate *in vitro* and/or *in vivo* in the absence of drug may be altered relative to wild type virus. However, a clear effect on disease pathology has not been reported.

In principle, however, the adoption of resistance mutations in an optimized, wild-type virus should result in reduced capacity for replication in the absence of drug, although relatively greater replication in the presence of drug. Although GCV-sensitive and -resistant infections could not be distinguished on the basis of cytopathology, Erice *et al.*, 1997⁴² have reported a GCV-resistant isolate with the UL54 mutation, T700A, which has previously been associated with a “slow-growth” phenotype based on plaque size and infectious virus yield of recombinant viruses.

Alteration of viral properties by resistance mutations to enhance *in vitro* replication has also been observed. A clinical isolate containing the UL54 substitution L501F, has been found to replicate faster *in vitro* and to be released more efficiently into culture medium than the wild type.⁴³ This apparent improvement of growth *in vitro* may result in an *in vivo* disadvantage to the virus in the absence of drug, otherwise it would be expected that the mutant form would predominate as wild-type. As noted earlier, however, more serious research is needed to define the role of drug-resistant mutants in the pathology of disease processes.

C.2f. Prevalence of Resistance in Clinical CMV Isolates

The sponsor has cited a number of reports documenting the presence of GCV resistant mutants in immunocompromised patients. However, there is no systematic epidemiologic survey yet to accurately document the prevalence of GCV resistance in the untreated or even treated AIDS population. The sponsor has, however, summarized that the prevalence of CMV resistance to GCV in individuals with CMV retinitis who have not previously been treated with GCV appears to be uncommon and varied from 0.9% to 2.7% of the patient population. As has been recognized, these data may not be conclusive as more standardized protocols for specimen collection, storage and virus sensitivity assay should be followed by all the clinical centers with regard to the prevalence of CMV resistance in patient populations undergoing treatment with GCV.

C.2g. Incidence and Frequency of CMV Resistance Following Treatment with GCV

HCMV strains resistant to ganciclovir have also been isolated from immunocompromised patients who received prolonged courses of the drug. Ordinarily these studies have compared the susceptibilities of the pre- and post-therapy isolates to GCV in cell culture *in vitro*. Drew *et al.*,

(1991)⁴³ first reported that the incidence of CMV resistance following treatment with intravenous GCV may be approximately 8%. More recently, it was reported that in a sequential cohort of 76 patients, resistant virus isolates (blood or urine of patients, $IC_{50} > 6.0 \mu M$) were found in 11.4% after 6 months of GCV treatment and in 27.5% after 9 months of GCV treatment.⁴⁴

Theoretically oral GCV has the greater potential to induce viral resistance than IV GCV. Because of its relatively poor bioavailability, oral doses as high as 3000 mg per day have resulted in a lower peak serum concentration ranging from 0.2 -1 $\mu g/ml$, while an IV therapy of 5 mg/kg/day may yield a maximum concentration of 8.0 $\mu g/ml$ of GCV. Since the IC_{50} values for most of the clinical isolates have ranged from 0.02 to 6.48 $\mu g/ml$ emergence of GCV resistant mutants is more likely after oral administration. Drew *et al.*, (1999)⁴⁵ have recently reported that about 3.1% of patients receiving IV GCV and 6.5 % receiving oral GCV shed resistant HCMV (median GVC exposure of 75 and 165 days, respectively). Therefore, the sponsor has concluded that the development of resistance was unaffected by choice of GCV formulation.

However, many clinical investigators have cautioned that the incidence of real GCV resistance may continue to be underestimated as long as the collection and storage of specimens, cell culture and phenotyping methods are not standardized to yield consistently reproducible sensitivity test results from various clinical studies. Equally important is the assumption that GCV resistant CMV isolates must be able to replicate several passages in culture may introduce several variables to the data collection and interpretation of the results. In case the mutant fails to grow for several passages *in vitro*, the culture is usually considered a failure or negative, rather than indicative of viral resistance. More rationale research efforts should be directed to address these shortcomings.

C.2h. Diversity of CMV Strains and Ganciclovir Resistance in Individual Patients

immunocompromised patients, especially those with AIDS may harbor different CMV strains. Multiple strains of virus have been identified in clinical isolates from different organs of the same patient.⁴⁶ Although viral isolates from such diverse body fluids as CSF and urine can display identical mutations, mutant viral strains can also differ between eyes in individuals with bilateral retinitis.³⁵ Clearly, clinical isolates comprise a number of different viral variants with different properties. Disparity between CMV genotypes and phenotypes in different organs could have substantial implications for alterations in therapy and may provide insight into the local organ specific environment with respect to CMV replication and effectiveness of antiviral drugs.

The differences in resistance profiles in individual patients and the implications have been discussed by Emery.⁴⁷ In an AIDS patient, resistance mutations in the UL97 gene at codons 460, 594 and 595 were investigated in multiple organs obtained after autopsy. Resistance at codon 595 was seen in CMV present in the duodenum, pancreas and stomach, whereas in other organs, all CMV detected was wild type at these codons. This factor in the resistance profile of CMV strains should be considered when assessing the CMV resistance status of individual patients based on blood samples.

Thus there is the possibility that a complex mixture of CMV phenotypes isolated from patients during cell culture *in vitro*, may or may not represent the CMV genotypes *in vivo*. As a result the *in vitro* sensitivity of a CMV strain from urine samples may not be the same CMV replicating and causing disease in the retina. A systematic survey of the CMV strains isolated from various test organs and body fluids should address the question related to differential sensitivity of various clinical isolates in immunocompromised patients.

D. CLINICAL VIROLOGY

Clinical study WV15376 was the only study exclusively designed to compare the safety and efficacy of VGCV, vs. IV GCV as induction therapy for the treatment of newly diagnosed peripheral CMV retinitis. However, brief descriptions of a number of clinical studies demonstrating the efficacy and safety of GCV (both IV and oral formulations) in the treatment and prevention of a number of CMV diseases are also included in this submission. The sponsor has stated that the antiviral effect of ganciclovir has been observed in randomized, controlled clinical trials (1257, 1288, 1774, 1653, 226, 2304) in patients with documented CMV disease and/or active CMV infection. In addition, six randomized, controlled clinical trials (studies 1570, 1689 1496, 040, 2681 and 1654) have shown that both IV and oral GCV were effective agents for the prevention of CMV disease in asymptomatic individuals. These studies are listed and very briefly described in Table 9.

Table 9. Description of clinical studies in the ganciclovir clinical virology summary

Study	Description
2304	A randomized controlled study of the safety and preventative efficacy of oral ganciclovir when used in conjunction with intravitreal ganciclovir implant in the treatment of cytomegalovirus retinitis. 2000.
2681	A study of the safety and efficacy of oral ganciclovir for the prevention of CMV infection in bone marrow transplant recipients. 1998.
040	A multicenter, double blind, randomized, placebo controlled study of the efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease in liver transplant recipients. 1996.
2226	A randomized study comparing the safety and efficacy of three doses of oral ganciclovir to intravenous ganciclovir for the maintenance treatment of retinitis in people with AIDS. 1996.
1654	A randomized double blind study of the efficacy and safety of oral ganciclovir for the prevention of CMV disease in people infected with the human immunodeficiency virus. 1995.
1653	A randomized controlled study of the efficacy and safety of maintenance treatment with oral ganciclovir for newly diagnosed cytomegalovirus retinitis in people with AIDS. 1994.
1774	A randomized study comparing the safety and efficacy of two regimens of oral ganciclovir to intravenous ganciclovir maintenance therapy for cytomegalovirus retinitis in people with AIDS who have received prior ganciclovir therapy. 1994.
1288	A double blind, placebo controlled study of intravenous ganciclovir (DPHG) for cytomegalovirus colitis in patients with acquired immune deficiency syndrome. 1991.
1496	A randomized, double blind, placebo controlled study of prophylactic intravenous ganciclovir in heart transplant patients with CMV at risk of developing pneumonia. 1991.
1570	Ganciclovir, 9-(1,3 dihydroxy-2-propomethyl)guanine, in the prevention of cytomegalovirus pneumonia in allogeneic bone marrow graft recipients with hematologic malignancies. 1991.
1689	A randomized, double blind, placebo controlled study of prophylactic intravenous ganciclovir in bone marrow transplant patients (BMT) with cytomegalovirus infection. 1991.
1257	Ganciclovir (DPHG): Controlled clinical study of induction treatment in patients with life or sight threatening cytomegalovirus infection. 1988.

The results of a number of these studies have been reviewed earlier for NDAs appropriate for the already approved IV or oral formulations of GCV. The salient feature of the virology data reviewed earlier may be summarized as follows. In most of the clinical centers, samples of blood, urine, semen or throat wash were collected for CMV culture and determination of viral shedding and susceptibility to GCV. In some centers CMV DNA load in the plasma was quantitated for the evaluation of efficacy of GCV. According to the sponsor, the results from these studies provided evidence that there was a relatedness of the virus load, as measured by either virus culture or PCR, to the progression of CMV disease, and that the incidence of GCV resistance was quite low (6.7%). However, as reviewed earlier, there were a number of problems with the virology data and interpretation of the results presented.

First, there were a few subjects who were CMV culture positive while undergoing GCV treatment and therefore, a very small sample of CMV isolates were available for testing. In addition, the protocols for virus collection, assay and interpretation of the results were not standardized. While the clinical studies required that CMV cultures be tested according to the schedule, there was no specific protocol that could have been uniformly used by all the clinical centers for appropriate comparison of the results. Thus, the virus isolation procedures ranged from shell vial culture in one clinical laboratory to a conventional plaque assay method in another laboratory using unspecified cell lines. It is also not known how many serial passages of the virus isolates had to undergo before it was determined that a particular sample was "culture positive." Because of the lack of uniformity and standardization of these basic virologic methods, the results obtained on viral shedding and resistance from these studies are not interpretable.

Compounding these issues were additional problems with the sensitivity testing of the clinical samples. The sponsor had indicated that not all culture positive isolates could be tested for sensitivity and the frequency of resistance in untested isolates remained unknown. As discussed earlier, there were some incongruities in the results of susceptibility testing as well. Some patients in the studies were shedding a sensitive strain on day 56, a resistant strain on day 112, and a sensitive strain again on day 125 while still under treatment. Another patient in the same study was shedding a resistant strain of CMV on day 1 and a sensitive isolate on day 30. While these inconsistencies in the susceptibility test results are extremely difficult to explain, it obviates the fact that systematically well designed experimental approach in the collection, storage and virus assay may provide more reliable results on the incidence of CMV resistance in any given population.

Clinical Study WV15376

The protocol was an open label, parallel group, multicenter, randomized study designed to test the hypothesis that VGCV 900 mg b.i.d. would result in clinically satisfactory induction therapy in patients with newly diagnosed peripheral CMV retinitis. More specifically, the study was designed to evaluate the safety and efficacy of oral VGCV (900 mg b.i.d. for 3 weeks followed by 900 mg o.d. for one week) vs. IV GCV (5mg/kg b.i.d. for 3 weeks followed by 5mg/kg o.d. for one week). A total of 160 HIV seropositive patients with newly diagnosed CMV retinitis were enrolled in 42 clinical centers world wide. After completing the first 4 weeks of randomized treatment patients were able to receive VGCV maintenance therapy (900 mg o.d.) in an extension of the study. If progression of CMV retinitis was diagnosed during the extension phase, re-induction therapy with VGCV (multiple cycles) were initiated.

The objectives of the study were:

1. To investigate the efficacy of VGCV when used as induction therapy in patients with newly diagnosed CMV retinitis

2. To investigate the safety profile of VGCV in this indication
3. To assess the effects of induction and maintenance level dosing of VGCV on CMV viral load, measured by CMV-PCR
4. To measure the pharmacokinetics of GCV following VGCV administration.

A virology substudy was also undertaken as part of this protocol WV15376, in order to investigate directly the antiviral activity of VGCV. Virus cultures were used to detect and isolate CMV shedding in urine and to detect CMV in the blood of patients. One clinical study site also performed CMV cultures of semen samples. Qualitative and quantitative PCR analyses were performed to detect and quantify viral DNA in plasma.

1. Urine cultures were performed at Screening and Week 4 in the current study in order to investigate the differences between ganciclovir and valganciclovir after the induction period. This would allow the direct effect of each drug on viral shedding to be investigated, and enable comparisons to be made with previous ganciclovir studies (1288 report CL5776, 1570 report CL5557, 1689 report CL5567, 2304 report [in preparation]) [8021, 8071, 8072, 8017].

2. Additional cultures were performed at 3 and 6 months, during the maintenance phase, as these were timepoints known to be associated with the emergence of ganciclovir resistance.

3. Qualitative and quantitative CMV PCR was performed at every visit to demonstrate the direct antiviral effect of treatment with ganciclovir or valganciclovir, seen as a reduction in viral load or a change from PCR positive to negative, during induction and maintenance dosing. In addition it is also potentially possible to address several related questions such as whether PCR is predictive of disease progression and if an increase in viremia is predictive of a risk of the development of resistance.

Although the quantitative PCR method used in this study is not yet approved by the FDA for clinical use, the availability of blood cultures at every visit provide a comparative sample from the same compartment. Additionally, HIV PCR analysis was performed to determine the HIV status of patients enrolled in the study and as an indicator of whether the HIV burden in the patients was successfully managed by their antiretroviral therapy.

For the virologic endpoints the following procedures were followed.

1. CMV Culture

From Urine Samples

Urine samples for CMV cultures were obtained at Screening (CMV treatment naïve) and at Week 4 to investigate the antiviral effect of VGCV and GCV induction therapy. Additional samples were taken at 3 months, 6 months and at study termination. Details of the urine sample processing for CMV cultures are described in Appendix 1. Briefly, a midstream urine sample was collected in a sterile urine collection cup and sent immediately to the local virology laboratory for processing. Samples from study sites, which were unable to perform CMV cultures locally, were placed in M4 transport medium, cooled, and shipped immediately to the nearest _____ laboratory for processing. The _____ processed the samples according to the methods described in Appendix 1, within 48 hours of collection. All isolates obtained were tested for ganciclovir sensitivity.

As conventional cultures require two weeks to a month before results may be obtained, in this study two shell vial cultures were established from each urine specimen. After 48 hours, one shell vial culture was stained using local laboratory procedures to detect CMV. If it was negative, the patient was deemed to be CMV culture negative and the second shell vial was destroyed. If the first shell vial was positive, the second vial was expanded to conventional culture and an isolate was frozen for ganciclovir sensitivity testing.

From Blood Samples

Blood samples for CMV culture were collected at every visit (at screening, weekly to Week 4, every second week to first progression of CMV retinitis or Week 16, then monthly and at termination). The sponsor has noted that only a small numbers blood samples were collected and evaluated because this test procedure (CMV culture from blood samples) was introduced late into the study, when half of the patients had already entered this study.

Study sites that were not able to perform CMV cultures locally collected the blood samples as described above, then shipped the samples (cooled) immediately to the _____ for processing. The _____ processed the samples according to the methods described above and in Appendix 1, within 48 hours of collection.

From Semen Samples

Only one clinical trial site prepared semen cultures according to their 'in house' protocol. This involved seeding the seminal fluid onto a monolayer of low passage normal human dermal fibroblasts (NHDF). CMV cultures were maintained in a standard fibroblast culture medium.

2. CMV PCR Analysis

Plasma samples for qualitative and quantitative CMV PCR were obtained at each study visit _____ . Neither of these two tests have been approved by FDA.

The qualitative and quantitative plasma CMV PCR tests designed by Roche Molecular Systems Inc (RMS) were used in study WV15376 because they use standardized technology (_____). The RMS _____ technology is well established in the field of HIV, being used for HIV detection and monitoring the efficacy of treatment. This technology has now been applied to CMV; the qualitative CMV PCR test has been clinically validated, however the quantitative test has not yet been fully validated in the clinical setting.

The CMV PCR analysis was performed in collaboration with RMS. RMS was responsible for the development of the PCR protocols (described in Appendix 2 and Appendix 3), training of the laboratory personnel, certification of the PCR laboratories and for ensuring the quality of the _____ data.

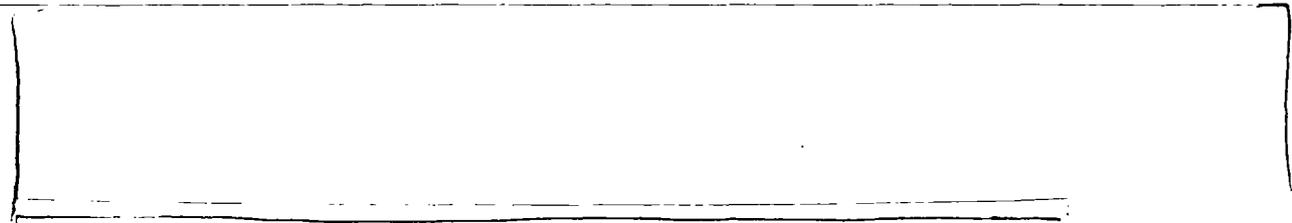
The CMV PCR analysis was undertaken at one of three independent laboratories appointed for the study: _____

Blood samples were taken from each patient at each study visit, (Screening, weekly for the first 4 weeks, every second week until first progression or Week 16, monthly thereafter and at termination).

The plasma was removed and the samples frozen at the study site. Frozen samples were transported on dry ice to the _____ for storage at -70°C . Samples were aliquoted for analysis and shipped by _____ to the other laboratories for analysis.



Qualitative Plasma CMV PCR Analysis

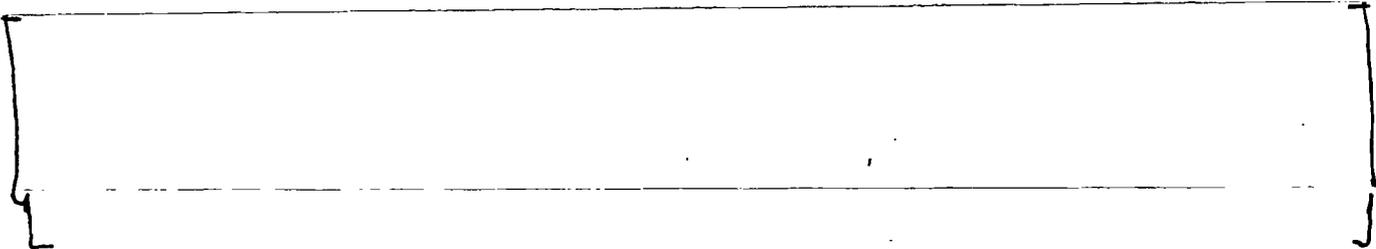


Peripheral Mononuclear Leukocyte CMV PCR Analysis

To document the emergence and nature of GCV resistance, genotypic analysis of the UL97 gene of CMV obtained from the peripheral blood leukocyte samples patients enrolled in this study has been undertaken. This analysis included amplification by PCR of regions of the CMV genome known to be associated with ganciclovir resistance.

Blood drawn at each visit from patients enrolled in this study was sent to the nearest appointed _____ Here, the peripheral blood leukocytes were extracted and divided into aliquots of 10^6 cells/vial and frozen at -70°C . Aliquots of frozen lymphocytes were shipped to _____ laboratory on dry ice for analysis.

CMV Analysis



HIV Analysis

HIV load was measured by _____ in plasma samples taken from all patients at Screening and Week 1, at Week 4, every 6 months and at Termination. As most patients enrolled in study WV15376 were receiving protease inhibitors (PI), the HIV burden was measured initially by the ultrasensitive _____ (1.5). This test has a linear range of _____ Those samples with an HIV burden above the linear range of this assay

were retested with the standard [7776] test with a linear range of [7776].

The plasma samples used for the analysis were collected and stored as described for the CMV samples above. Quantitative HIV analysis was performed using two [7776]. The [7776] allows the automation of HIV DNA amplification, detection and quantification in the routine laboratory setting. The HIV analysis was carried out at the [7776]. The HIV plasma samples were also stored at [7776] instead of the recommended -70°C. It is therefore possible that this could have led to a reduction in the measurable HIV loads for some samples.

Results

In order to present the results the sponsor has defined the patient populations as follows.

Two patient populations were defined for efficacy analysis and summaries, intent-to-treat and standard population. Data for the intent-to-treat (ITT) population is presented in this virology substudy report. The ITT population included all patients who were randomized (n=160). Patients who received incorrect therapy compared to that intended, or who did not receive any therapy, were included in the group to which they were randomized. The tables included in the text of this report include analyses conducted on the ITT population.

CMV Culture Data

Table 10. Summary of CMV cultures at screening¹

	GCV/VGCV N = 80	VGCV/VGCV N = 80
URINE CMV CULTURE		
POSITIVE	45 (64%)	32 (46%)
NEGATIVE	25 (36%)	37 (54%)
NOT KNOWN	10	11
SEMEN CMV CULTURE		
POSITIVE		2 (100%)
NEGATIVE	1 (100%)	
NOT KNOWN	79	78
BLOOD CMV CULTURE		
POSITIVE	13 (46%)	5 (20%)
NEGATIVE	15 (54%)	20 (80%)
NOT KNOWN	52	55

A patient is recorded as positive if they return a positive result for at least one of the tests for Urine, Blood or Semen. If no positive tests occur then they are similarly recorded as negative if they have at least one negative test result for Urine, Blood or Semen.

Percentages are calculated using the total number of negative and positive results for the treatment group & visit.

¹From Table 1, Vol. 84-19

At Screening, 64% of patients in the GCV arm and 46% of those in the VGCV arm who had urine cultures performed were positive for CMV. At the one study site where semen cultures were undertaken, 2 patients in the VGCV arm (and none in the GCV arm) were positive. Of those who had blood cultures, 46% in the ganciclovir arm and 20% of those in the VGCV arm were positive. 'Not known' refers to those patients for whom there was no CMV culture result due to non-collection, loss of the sample due to incorrect sample handling, or poor culture technique.

After 4 weeks treatment, at the Week 4 visit, the incidence of positive CMV urine cultures decreased to 6% (4/62) in the ganciclovir arm and 7% (4/55) in the valganciclovir arm of the study (Table 11). The sponsor has concluded that these results represent a significant reduction in viruria in patients treated with either GCV or VGCV

After Week 4, urine cultures were performed for relatively few patients. Two patients (1102 and 3701) had positive urine cultures at one or more visits after Week 4. Of the 7 patients with positive urine cultures at Week 4, only one (903) had urine culture performed after Week 4 (on Day 87), at which time both culture and PCR were negative.

Table 11. Summary of urine culture data at screening and Week 4¹

CMV Urine Cultures (ITT Population)

Visit	GCV/VGCV	GCV/VGCV	GCV/VGCV	VGCV/VGCV	VGCV/VGCV	VGCV/VGCV
	POSITIVE N = 80	NEGATIVE N = 80	NOT KNOWN N = 80	POSITIVE N = 80	NEGATIVE N = 80	NOT KNOWN N = 80
SCREENING	45 (64%)	25 (36%)	10	32 (46%)	37 (54%)	11
WEEK 4	4 (6%)	58 (94%)	18	4 (7%)	51 (93%)	25

Percentages are calculated using the total number of negative and positive results for the treatment group & visit
¹From Table 2, Vol 84-20

Table 12. Summary of blood culture data at screening and Week 4¹

CMV Blood Cultures (ITT Population)

Visit	GCV/VGCV	GCV/VGCV	GCV/VGCV	VGCV/VGCV	VGCV/VGCV	VGCV/VGCV
	POSITIVE N = 80	NEGATIVE N = 80	NOT KNOWN N = 80	POSITIVE N = 80	NEGATIVE N = 80	NOT KNOWN N = 80
SCREENING	13 (46%)	15 (54%)	52	5 (20%)	20 (80%)	55
WEEK 4	20 (100%)	60	23 (100%)	57		

Percentages are calculated using the total number of negative and positive results for the treatment group & visit
¹From Table 3, Vol 84-20

In Table 12, it can be seen that 13 (46%) of patients in the ganciclovir arm and 5 (20%) in the valganciclovir arm had positive blood cultures at Screening. Of these 18 patients, only one had a negative urine culture at Screening. No patient had a positive blood culture at Week 4. No patients were positive at any visit after Week 4. Blood cultures were introduced into this protocol by amendment E (the protocol was finalized on April 22, 1998). The low numbers of patients with blood cultures performed at Screening reflect the late introduction of this test into the study (after about half the patients had enrolled). Of the 7 patients with positive urine culture at Week 4, only two had blood cultures performed; patients 2218 and 3802 had positive blood cultures at baseline which were negative by Week 4.

One study site performed semen cultures in 3 patients. The single patient tested in the ganciclovir arm was negative at Screening. Two patients in the valganciclovir arm were positive at Screening and one at Week 4. The patient that was positive at Week 4 did not have a urine culture at that visit but was blood culture negative.

CMV PCR Analysis

Qualitative PCR

Results presented in Table 6, Vol. 84-22 show that in the GCV arm of the study 39 (51%) of patients were PCR positive for CMV in plasma at Screening and by Week 4, this had decreased to 2 (3%).

In the VGCV arm, 31 (40%) patients were CMV PCR positive at Screening, this decreased to 3 (4%) at Week 4. After Week 4 (when all subjects received VGCV), at any one visit, only a few (maximum of 4 (11%)) in either treatment group were PCR positive for CMV at any one visit. This remained true through the duration of the study. However, it must be mentioned that as the study progressed the number of patients that were tested by PCR declined and by Extension Visit 10, only 37% of patients were tested and by Extension Visit 20, only 11% of patients were tested.

Comments: 1. The sponsor has concluded that that induction therapy with either IV GCV or oral VGCV leads to a marked decrease in viruria and viremia 4 weeks after initiation of therapy. This anti-viral response is sustained by VGCV maintenance therapy. However, the number of patients at study entry in each arm were limited, and there was an imbalance in the proportion of patients in the randomized groups who were shedding CMV in the urine at study entry; 64% in the IV. GCV group and 46% in the VGCV group. Virologic status of these patients, however, is not known.

2. A number of patients were on therapy with protease inhibitors. The influence of protease inhibitor on the antiviral activity of GCV remains to be elucidated.

3. A very limited number of patients (13 in GCV arm and 5 in the VGCV arm) had positive CMV cultures in the blood samples collected at screening. Significance of the results obtained from such a low number may not be taken too seriously.

4. Culture positive urine, blood or semen samples could be obtained from only a low proportion of patients during the maintenance period. As the sponsor has suggested, this could reflect a very low CMV viral load in study patients resulting from effective GCV/VGCV therapy and VGCV maintenance. However, it is also likely that the lack of positive cultures may be due to poor laboratory technique of collection, and virus assay procedures.

5. The _____ test was used for qualitative PCR and the _____ FDA is yet to approve these tests.

6. The majority of the plasma samples for CMV PCR analysis had been stored erroneously at _____ at _____ instead of -70°C . While the effects of long term storage at -20°C are unknown, the validity of the results obtained on such samples through CMV PCR data analysis and the interpretation of such results may not be accurate.

7. Some patients such as 2211, 3701, 3802 (Appendix 4) had plasma viral load of 10^3 copies/ml, but the blood culture assay was negative. On the hand there are instances where plasma PCR analysis was negative, but the blood and urine culture was positive (patients 407, 409, 701, 1302, Appendix 4).

8. There are other inconsistencies as well. Patient 401 who was CMV negative at screening, became CMV positive after one week of therapy. Patient 904 was positive at screening, became negative at Week 2 to become positive and negative in a cyclical fashion.

E. SUMMARY AND CONCLUSIONS: 1. In this NDA the sponsor is seeking approval of a 450 mg tablet formulation of VGCV to improve bioavailability, compliance and ease of oral administration by immunocompromised AIDS patients with CMV retinitis. VGCV is the valyl ester prodrug that is rapidly metabolized to GCV. The antiviral properties of VGCV are stated to be those of GCV and oral administration of VGCV may provide GCV exposures equivalent to those achieved only with the IV formulation.

2. The CMV encoded protein kinase, pUL97, monophosphorylates GCV in CMV- infected cells. After additional phosphorylation by cellular kinases to its di-, and tri-phosphate forms, GCVTP inhibits viral DNA replication by (i) competitive inhibition of viral DNA pol, and (ii) may direct incorporation into viral DNA resulting in slow termination of nascent viral DNA chain elongation.

3. The potency of GCV to inhibit various laboratory strains and clinical isolates of human CMV has been found to be dependent upon a number of factors including the host cells, virus strain, multiplicity of infection, assay method, and even the personnel performing the test. Thus, the concentration of GCV that inhibits replication of various strains of CMV has ranged from 0.02 to 3.48 µg/ml. However, the relationship between the *in vitro* sensitivity of CMV to GCV and clinical response has not been established.

4. GCV inhibits mammalian cell proliferation *in vitro* with IC₅₀ values ranging from 30 to 725 µg/ml. However, bone marrow-derived colony-forming cells were highly sensitive with GCV IC₅₀ values ranging from 0.028 to 0.7 µg/ml. These results indicate that GCV may be quite toxic to mammalian cells of hematopoietic origin.

5. Because of high species specificity, HCMV does not grow well in laboratory animals. Although a number of related CMV-infected animal models have been used to mimic the human infection *in vivo*, the choice of animal models, virus strain, mode of treatment, and time of initiation of treatment have been quite variable and all influenced the effectiveness of treatment. In addition, the relevance of animal models in predicting clinical efficacy in human subjects has not been determined.

6. Laboratory strains and clinical isolates of human CMV have demonstrated significantly variable susceptibility to GCV, some of which may be resistant to >1.5 µg/ml of GCV. GCV resistant mutants carry mutations in UL97, UL54, or both the genes of CMV.

7. Results from a number of clinical studies indicate that (i) the prevalence of CMV resistance to GCV in AIDS patient population who have not been treated with GCV may be uncommon (<2% of the patient population), and (ii) the incidence of CMV resistance following treatment with GCV have ranged from 8 to 15% of this population. However, these results were based upon a very low sample size, and gross inconsistencies in the sensitivity test protocols including sample collection, virus isolation, and virus assay. Clinical significance of the GCV-resistant mutants remains to be elucidated.

9. Since the two viral genes, CMV UL97 gene and the CMV UL54 gene encoding the viral DNA pol, are known to interact with GCV and its GCV-TP, respectively, mutations in these two viral genes have also been identified and characterized to be responsible for HCMV resistance to GCV.

10. Data collected in one clinical study WV15376 on viruria (CMV urine culture), and viremia (CMV blood culture and PCR analysis) have demonstrated that VGCV has the potential of an anti-CMV agent in patients with CMV retinitis. However, the conclusions that oral VGCV lead to "marked decrease" in viruria and viremia is not based on solid experimental evidence. Only a limited number of samples were collected, virus assay protocols were not standardized, erroneously stored at higher temperature (-20 C vs. -70 C) and used PCR assay procedures yet to be validated by FDA.

11. The NDA is approvable with respect to microbiology. The microbiologic issues in the package insert for VGCV 450 mg tablets must be revised as recommended.

REFERENCES:

1. Roizman B., Carmichael L.C., Deinhardt F., *et al.*, Intervirology, 1981, 16:201-217
2. Alford C.A. and Britt W.S., In Roizman B., Whitley R and Lopez C., Ed. The Human Herpesviruses, Raven Press Ltd., NY. 1993, pg 227-255.
3. Grant S., Edmond E., Syme, J., J. Infect. 1981, 10:257-264.
4. Wright H.T. Jr., Goodheart C.R., Leilausis A., Virology, 1964, 23:419-424.
5. Lakeman A.D. and Osborn J.F., J. Virol., 1979, 30:414-416.
6. Chee M.S., Bankier, A.T., Beck S., *et al.*, Current Topics Microbiol. and Immunol., 1990, 154:125- 170.
7. Stinski M.F., Malone C.C., *et al.*, In Wagner E.K., Ed., Herpesvirus Transcription and Its Control, Boca Raton, CRC Press, 1991, pg 241-260.
8. Kouzaridas T *et al.*, J.Virol, 1987, 61:125-123.
9. Pari, G.S. and Andres, D.G., J.Virol, 1993, 67:6979-6988.
10. Shepp D.H., *et al.*, Ann Intern. Med., 1985, 103:368-373
11. Wong S.W., *et al.*, EMBO J., 1988, 7:37-47.
12. Sullivan V., *et al.*, Antimicrob Agents Chemother., 1993, 37:19-25
13. Hwang C.C.,*et al.*, J. Virol, 1992, 66:1774-1776.
14. Gibbs, J.S., *et al.*, Proc. Natl. Acad. Sci, USA, 1988, 85:6672-6676.
15. Ye L.B., and Huang E.S. J Virol. 1993, 67:6339-6347.
16. Littler, E., *et al.*, Nature, 1992, 358: 160-161.
17. Sullivan, V., *et al.*, Nature,1992, 358: 162-164.
18. Michel, D., *et al.*, J. Virol,1996, 70:6340-6346.
19. Michel, D., *et al.*, J. gen. Virol. 1998, 79:2105-2112.
20. Huse, M., *et al.*, Cell, 1999, 96:425-436.
21. Michel, D., *et al.*, J. Virol. 1999, 73:8898-8901
22. Zimmerman, A., *et al.*, Antivir. Res., 1997, 36:35-42.
23. Smee, D.F., *et al.*, Biochem Pharmacol. 1985, 34:1049-1056.
24. Biron, K.K., *et al.*, Proc.Natl. Acad. Sci., USA. 1985, 82:2473-2477.
25. Medina, D.J., *et al.*, Antimicrob Agents Chemother., 1992, 36:1127-1130.
26. Freitas, V.R., *et al.*, Antimicrob Agents Chemother., 1985, 28:240-245.
27. Smee, D.F., *et al.*, Antimicrob Agents Chemother., 1992, 36:1837-1842.
28. Brown, J.M., *et al.*, J. Inf. Dis.1995, 171:1599-1603.
29. Fong, C.K.Y., *et al.*, Antivir Res., 1987, 7:11-23.
30. Biron, K.K., *et al.*, Proc. Natl. Acad. Sci. USA., 1986, 83:8769-8773.
31. Drew W.L., J. Am. J. Health Syst. Pharm., 1996, 53(suppl. 2):S17-23.
32. Lipson, S.N., *et al.*, Diagn. Microbiol. Inf. Dis., 1993, 17:283-91.
33. Lipson, S.N., *et al.*, Diagn. Microbiol. Inf. Dis., 1997, 28:123-129.
34. Chou, S., J. Infec. Dis., 1990, 162:738-742.
35. Liu, W., *et al.*, J. Infect. Dis., 1998, 177:1176-1181.
36. Lurain, N.S., *et al.*, J. Virol., 1992, 66:7146-7152.
37. Hanson, M.N., *et.al.*, Antimicrob Agents Chemother., 1995,39:1204-1205
38. Tatti, K.M., *et al.*,Intern.Antivir. News., 6., 1998.
39. Cihlar, T., *et al.*, J. Virol., 1998, 72:5927-5936.
40. Chou, S., *et al.*, Antimicrob Agents Chemother.,1999,43:1500-1502.
40. Tatarowicz, W.A., *et al.*, J. Infect. Dis., 1992, 166:904-907.
41. Eric, A., *et al.*, J. Infect. Dis., 1998, 178:531-534.
42. Harada, K., *et al.*, Arch. Virol., 1997, 142:215-225.

- 43. Drew, W.L., *et al*, J. Infect. Dis, 1991,163:716-719.
- 44. Jabs, D.A., *et al.*, Antimicrob Agents Chemother.,1998,42:2240-2244.
- 45. Drew, W.L., *et al*, J. Infect. Dis,1999,179:1352-1355.
- 46. Spector, S.A., *et al.*, J. Infect. Dis.,1984, 150:953-956.
- 47. Emery, V.C., Antivir. Ther., 1998, 3:239-242.

F. THE PACKAGE INSERT:

The VIROLOGY section of the label for VALCYT™ (Valganciclovir HCl) Tablets 450mg must be revised as follows.

VIROLOGY:

Mechanism of Action:

This subsection in the current label is written ((in italics below) as follows

Valganciclovir is an L-valyl ester salt (prodrug) of ganciclovir. Ganciclovir is a synthetic analogue of 2'-deoxyguanosine, which inhibits replication of _____ *in vitro and in vivo.*

In CMV-infected cells, ganciclovir is initially phosphorylated to ganciclovir monophosphate by the viral protein kinase, UL97. Further phosphorylation occurs by cellular kinases to produce ganciclovir triphosphate, which is then slowly metabolized intracellularly.

As the phosphorylation is largely dependent on the viral kinase, phosphorylation of ganciclovir occurs preferentially in virus-infected cells.

The reasons for the revisions in this subsection are as follows.

The recommended revisions to this subsection are as follows.

A. First paragraph

- 1. First two sentences.

Valganciclovir is an L-valyl ester (podrug) of ganciclovir that exists as a mixture of two

diastereomers. After oral administration, both diastereomers are rapidly converted to ganciclovir by intestinal and hepatic esterases.

1. Second sentence.

Ganciclovir is a synthetic analogue of 2'-deoxyguanosine, which inhibits replication of human cytomegalovirus *in vitro* and *in vivo*.

2. Third sentence must be deleted.

B. Second and third paragraph should be written as follows

In CMV-infected cells ganciclovir is initially phosphorylated to ganciclovir monophosphate by the viral protein kinase, pUL97. Further phosphorylation occurs by cellular kinases to produce ganciclovir triphosphate, which is then slowly metabolized intracellularly (half-life 18 hours). As the phosphorylation is largely dependent on the viral kinase, phosphorylation of ganciclovir occurs preferentially in virus-infected cells. The virustatic activity of ganciclovir is due to inhibition of viral DNA synthesis by ~~ganciclovir~~ triphosphate.

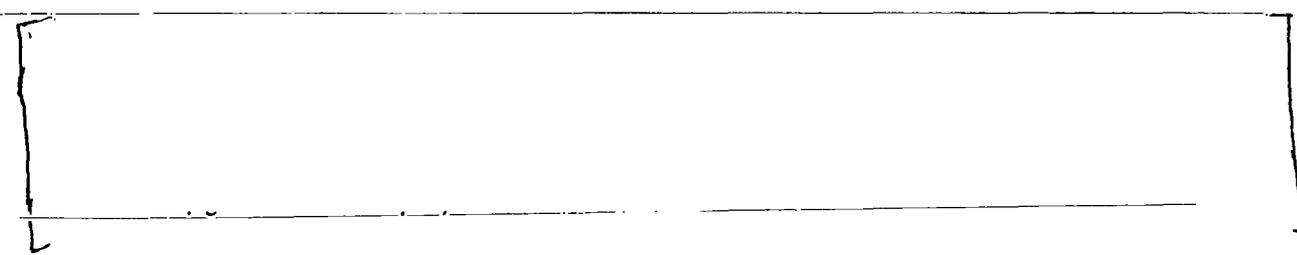
The new revised subsection on **Mechanism of Action** should read as follows.

Valganciclovir is an L-valyl ester (prodrug) of ganciclovir that exists as a mixture of two diastereomers. After oral administration, both diastereomers are rapidly converted to ganciclovir by intestinal and hepatic esterases. Ganciclovir is a synthetic analogue of 2'-deoxyguanosine, which inhibits replication of human cytomegalovirus *in vitro* and *in vivo*.

In CMV-infected cells ganciclovir is initially phosphorylated to ganciclovir monophosphate by the viral protein kinase, pUL97. Further phosphorylation occurs by cellular kinases to produce ganciclovir triphosphate, which is then slowly metabolized intracellularly (half-life 18 hours). As the phosphorylation is largely dependent on the viral kinase, phosphorylation of ganciclovir occurs preferentially in virus-infected cells. The virustatic activity of ganciclovir is due to inhibition of viral DNA synthesis by ~~ganciclovir~~ triphosphate.

Antiviral Activity:

This subsection in the current label is written as follows (in italics)



The subsection is to be revised as follows. The reasons are self-explanatory.

1. The following two sentences will precede the first sentence, which is also revised, of this subsection.

The quantitative relationship between the *in vitro* susceptibility of human [redacted] to antivirals and clinical response to antiviral therapy has not been established, and virus sensitivity testing has not been standardized. Sensitivity test results, expressed as the concentration of drug required to inhibit the growth of virus in cell culture by 50% (IC₅₀), vary greatly depending upon a number of factors.

2. The range of IC₅₀ values is to be revised on the basis of data presented in page 77, Appendix 2 of Vol. 83 of this NDA.

Thus the IC₅₀ of ganciclovir that inhibits human CMV replication *in vitro* (laboratory and clinical isolates) has ranged from 0.02 to [redacted] μg/mL (0.08 to [redacted] μM).

3. The last sentence of the subsection is to be deleted out.

The new revised subsection on **Antiviral Activity** should read as follows.

The quantitative relationship between the *in vitro* susceptibility of human herpesviruses to antivirals and clinical response to antiviral therapy has not been established, and virus sensitivity testing has not been standardized. Sensitivity test results, expressed as the concentration of drug required to inhibit the growth of virus in cell culture by [redacted] (IC₅₀), vary greatly depending upon a number of factors. Thus the IC₅₀ of ganciclovir that inhibits human CMV replication *in vitro* (laboratory and clinical isolates) has ranged from 0.02 to [redacted] μg/mL (0.08 to [redacted] μM). Ganciclovir inhibits mammalian cell proliferation (CIC₅₀) *in vitro* at higher concentrations ranging from 10.21 to >250 μg/mL (40 to > 1000 μM). Bone marrow-derived colony-forming cells are more sensitive (CIC₅₀ = 0.69 to 3.06 μg/mL: 2.7 to 12 μM).

Viral Resistance:

This subsection in the current label reads as follows (in italics).

Viruses resistant to ganciclovir can arise after _____ with valganciclovir by selection of mutations in either the viral kinase gene (UL97) responsible for ganciclovir monophosphorylation or in the viral polymerase gene (UL54). Virus with mutations in the UL97 gene is resistant to ganciclovir alone, whereas virus with mutations in the UL54 gene may show cross-resistance to other antivirals with a similar mechanism of action.

The current working definition of CMV resistance to ganciclovir in in vitro assays is $IC_{50} > 1.5 \mu\text{g/mL}$ ($6.0 \mu\text{M}$). CMV resistance to ganciclovir has been observed in individuals with AIDS and CMV retinitis who have never received ganciclovir therapy. Viral resistance has also been observed in patients receiving prolonged treatment for CMV retinitis with ganciclovir. The possibility of viral resistance should be considered in patients who show poor clinical response or experience persistent viral excretion during therapy.

A. The first sentence of the first paragraph of this subsection should be rewritten as follows.

Viruses resistant to ganciclovir can arise after prolonged treatment with valganciclovir by selection of mutations in either the viral protein kinase gene (UL97) responsible for ganciclovir monophosphorylation and/or in the viral polymerase gene (UL54).

B. The second paragraph must be deleted.

This paragraph does not add any new conclusive information.

The revised Viral Resistance subsection should be written as follows.

Viruses resistant to ganciclovir can arise after prolonged treatment with valganciclovir by selection of mutations in either the viral protein kinase gene (UL97) responsible for ganciclovir monophosphorylation and/or in the viral polymerase gene (UL54). Virus with mutations in the UL97 gene is resistant to ganciclovir alone, whereas virus with mutations in the UL54 gene may show cross-resistance to other antivirals with a similar mechanism of action.

The current working definition of CMV resistance to ganciclovir in in vitro assays is $IC_{50} \geq 1.5 \mu\text{g/mL}$ ($\geq 6.0 \mu\text{M}$). CMV resistance to ganciclovir has been observed in individuals with AIDS and CMV retinitis who have never received ganciclovir therapy. Viral resistance has also been observed in patients receiving prolonged treatment for CMV retinitis with ganciclovir. The possibility of viral resistance should be considered in patients who show poor clinical response or experience persistent viral excretion during therapy.

The new revised VIROLOGY section of the label should read as follows.

VIROLOGY

Mechanism of Action:

Valganciclovir is an L-valyl ester (prodrug) of ganciclovir that exists as a mixture of two diastereomers. After oral administration, both diastereomers are rapidly converted to ganciclovir by intestinal and hepatic esterases. Ganciclovir is a synthetic analogue of 2'-deoxyguanosine, which inhibits replication of human cytomegalovirus *in vitro* and *in vivo*.

In CMV-infected cells ganciclovir is initially phosphorylated to ganciclovir monophosphate by the viral protein kinase, pUL97. Further phosphorylation occurs by cellular kinases to produce ganciclovir triphosphate, which is then slowly metabolized intracellularly (half-life 18 hours). As the phosphorylation is largely dependent on the viral kinase, phosphorylation of ganciclovir occurs preferentially in virus-infected cells. The virustatic activity of ganciclovir is due to inhibition of viral DNA synthesis by ~~ganciclovir~~ triphosphate.

Antiviral Activity:

The quantitative relationship between the *in vitro* susceptibility of human herpesviruses to antivirals and clinical response to antiviral therapy has not been established, and virus sensitivity testing has not been standardized. Sensitivity test results, expressed as the concentration of drug required to inhibit the growth of virus in cell culture by 50% (IC₅₀), vary greatly depending upon a number of factors. Thus the IC₅₀ of ganciclovir that inhibits human CMV replication *in vitro* (laboratory and clinical isolates) has ranged from 0.02 to ~~0.08~~ μg/mL (0.08 to ~~0.08~~ μM). Ganciclovir inhibits mammalian cell proliferation (CIC₅₀) *in vitro* at higher concentrations ranging from 10.21 to >250 μg/mL (40 to > 1000 μM). Bone marrow-derived colony-forming cells are more sensitive (CIC₅₀ =

Viral Resistance:

Viruses resistant to ganciclovir can arise after prolonged treatment with valganciclovir by selection of mutations in either the viral protein kinase gene (UL97) responsible for ganciclovir monophosphorylation and/or in the viral polymerase gene (UL54). Virus with mutations in the UL97 gene is resistant to ganciclovir alone, whereas virus with mutations in the UL54 gene may show cross-resistance to other antivirals with a similar mechanism of action.

The current working definition of CMV resistance to ganciclovir in *in vitro* assays is IC₅₀ ≥ 1.5 μg/mL (≥ 6.0 μM). CMV resistance to ganciclovir has been observed in individuals with AIDS and CMV retinitis who have never received ganciclovir therapy. Viral resistance has also been observed in patients receiving prolonged treatment for CMV retinitis with ganciclovir. The possibility of viral resistance should be considered in patients who show poor clinical response or experience persistent viral excretion during therapy.

G. RECOMMENDATIONS:

1. The package insert must be revised according to the recommendations communicated earlier (See Section F above).

3. Clinical isolates of human CMV from individual immunosuppressed patients have already demonstrated variable degrees of susceptibility (or resistance) to GCV and there is a possibility that multiple strains of CMV may be responsible for various phenotypic expressions in a clinical setting. Therefore, sensitivity testing of CMV isolates from urine, blood or semen may not be truly representative of the CMV strains causing retinitis or certain other end organ CMV diseases. Please consider using most recent analytical methods to appropriately characterize various strains of CMV commonly isolated from immunocompromised patients undergoing oral VGCV treatment.

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CONCURRENCES:
HFD-530/ Assoc. Dir
HFD-530/TLMicro

CC:
HFD-530/Orig. NDA 21,304
HFD-530/Division File
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HFD-530/Review Micro
HFD-530/Stephens, RPM

**APPEARS THIS WAY
ON ORIGINAL**