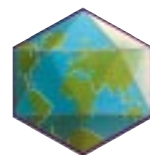


Recommendations from the  
IHMF Management Strategies Workshop  
and 4th Annual Meeting

Editors: Professor A Sivayathorn  
Professor RJ Whitley

# HERPESVIRUS INFECTIONS IN THE IMMUNOCOMPROMISED HOST WITH HIV- UPDATE



**IHMF**  
International  
Herpes Management  
— Forum —

# Participants

## CO-CHAIRS:

- Professor A Sivayathorn** Associate Professor of Dermatology,  
Department of Dermatology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
- Professor RJ Whitley** Professor of Pediatrics, Microbiology and Medicine,  
The University of Alabama at Birmingham, Suite 616 Children's Hospital, 1600 7th Avenue South,  
Birmingham AL 35233-0011, USA

## SPEAKERS:

- Dr Y Chang** Department of Neuropathology, Columbia University College of Physicians and Surgeons,  
630 West 168th Street, New York NY 10032, USA
- Dr J Feinberg** Associate Professor of Medicine,  
University of Cincinnati, Holmes Hospital, Eden and Bethesda Avenues, Cincinnati OH 45267-0405, USA
- Professor PD Griffiths** Professor of Virology,  
Division of Communicable Diseases, Department of Virology, Royal Free Hospital School of Medicine,  
University of London, Rowland Hill Street, London NW3 2PF, UK
- Dr MA Jacobson** Associate Professor of Medicine,  
San Francisco General Hospital, Building 80 Ward 84, 995 Potrero Avenue, San Francisco CA 94110, USA
- Professor RJ Whitley** Professor of Pediatrics, Microbiology and Medicine,  
The University of Alabama at Birmingham, Suite 616 Children's Hospital, 1600 7th Avenue South,  
Birmingham AL 35233-0011, USA
- Dr W Zhong** Howard Hughes Medical Institute, Department of Microbiology, University of California San Francisco,  
San Francisco CA 94143-0414, USA

## DISCUSSANTS:

- Dr AP Fiddian** Director of Viral Diseases,  
International Medical Affairs, Glaxo Wellcome Research and Development, Stockley Park, Uxbridge,  
Middlesex UB11 1BT, UK
- Dr S Kroon** Consultant Physician in Dermatology and Venereology,  
Department of Internal Medicine, Amtssygehuset Roskilde Kogevej 7-13, Post boks 247,  
DK-400 Roskilde, Denmark
- Dr D Pillay** Consultant Medical Virologist,  
Regional Virus Laboratory, Birmingham Public Health Laboratory, Birmingham Heartlands Hospital,  
Birmingham B9 5SS, UK
- Dr A Volpi** Researcher,  
Department of Public Health 135, University of Rome, Via di Tor Vergata, 00133 Rome, Italy
- Dr MJ Wood** Consultant Physician,  
Department of Infection and Tropical Medicine, Birmingham Heartlands Hospital, Bordesley Green East,  
Birmingham B9 5ST, UK

## ANNUAL MEETING SPEAKERS:

- Professor HH Balfour** Professor of Laboratory Medicine and Pathology,  
University of Minnesota, 516 Delaware Street, SE Minneapolis, MN55455-0392
- Dr P Moore** Division of Neuropathology,  
Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032, USA

The *International Herpes Management Forum* (IHMF) was established to improve the awareness, understanding, counselling and management of infections caused by herpesviruses. Steered by an IHMF Board of Professor Richard Whitley, Dr Martin Wood, Dr Larry Corey, Professor Paul Griffiths, Dr Susanne Kroon, Dr Antonio Volpi and Dr Koichi Yamanishi, the IHMF involves international opinion leaders in all aspects of medical management of herpesvirus infections.

The ninth IHMF workshop was held on 13 June 1996 to discuss herpesvirus infections in the immunocompromised host with HIV. This has been the topic of a previous workshop in June 1994 and a *Management Strategies in Herpes* publication. However, the wealth and breadth of research in this area has led to such significant improvements in both the treatment of herpesvirus infections, and our understanding of their pathogenesis in the HIV-infected individual so as to necessitate an update of the existing publication. The aim of the ninth IHMF workshop was therefore to expand and update existing management guidelines in light of current data and to develop guidelines in areas where they did not exist previously.

These draft recommendations were discussed at the 4th Annual Meeting of the IHMF that took place on 9–10 November 1996. This publication, *Herpesvirus Infections in the Immunocompromised Host with HIV – Update*, is part of the series, *Management Strategies in Herpes*. It contains amendments made to the guidelines following extensive discussion at the 4th Annual Meeting.

The editors would like to thank all the participants in the ninth IHMF workshop and those at the 4th Annual Meeting for their contribution and especially the Co-Chairs of the Working Groups.

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## Kaposi's Sarcoma-Associated Herpesvirus

### Evidence for a New Herpesvirus

#### Discovery

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus type 8, was first discovered in 1994.<sup>1</sup> Using a technique known as representational difference analysis,<sup>2</sup> two novel DNA fragments (KS330 and KS631) were isolated from the Kaposi's sarcoma lesions of individuals with AIDS, which were specific for the lesions.<sup>1</sup> These DNA sequences were shown to be of non-human origin and demonstrated homology to the gammaherpesvirus family (Epstein-Barr virus [EBV] and herpesvirus saimiri [HVS]), which characteristically replicate in lymphoblastoid cells.

#### The KSHV genome

Preliminary characterization of KSHV revealed a large episomal genome.<sup>3</sup> A 20.7 kb region of the genome has been completely sequenced and shown to have sequence and positional homology to known gammaherpesvirus genes, including the major capsid protein and thymidine kinase genes (Figure 1). Phylogenetic analyses comparing amino acid sequences of the major capsid protein of KSHV with a variety of other human as well as animal herpesviruses have demonstrated that the agent is a gamma 2 herpesvirus and is the first member of this genus known to infect humans.

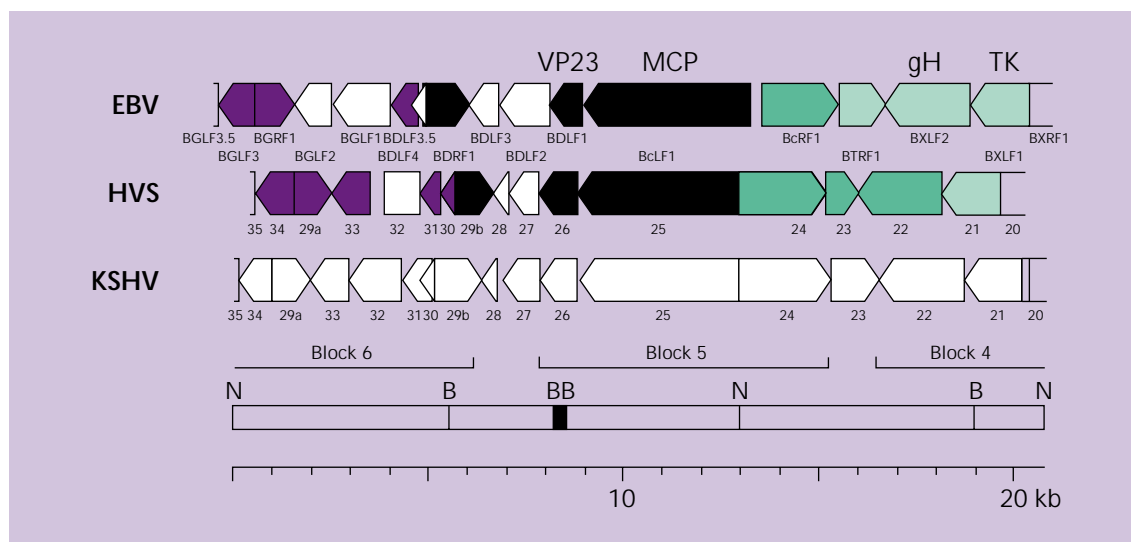


Figure 1: Partial genomic map of KSHV showing sequence homology to EBV and HVS<sup>3</sup>

The genomic size of KSHV is 160–165 kb.<sup>4</sup> Studies to determine which regions of the KSHV genome are actively transcribed found that gene expression in Kaposi's sarcoma is highly restricted and identified only two abundant transcripts of 1.1 kb and 0.7 kb, referred to as t1.1 and t0.7, respectively.<sup>5</sup> This suggests that most of the cells in the lesion are latently and not lytically infected by KSHV, consistent with the behaviour of herpesviruses generally. *In situ* hybridization studies confirmed that almost all Kaposi's lesions tested were positive for these two transcripts, the functions of which are not yet known.

## Morphology

The structure of KSHV has been visualized by electron microscopy and revealed the viral particles to have a nucleic acid core and capsid structure surrounded by an envelope (Figure 2), typical of herpesvirus morphology.<sup>6</sup>

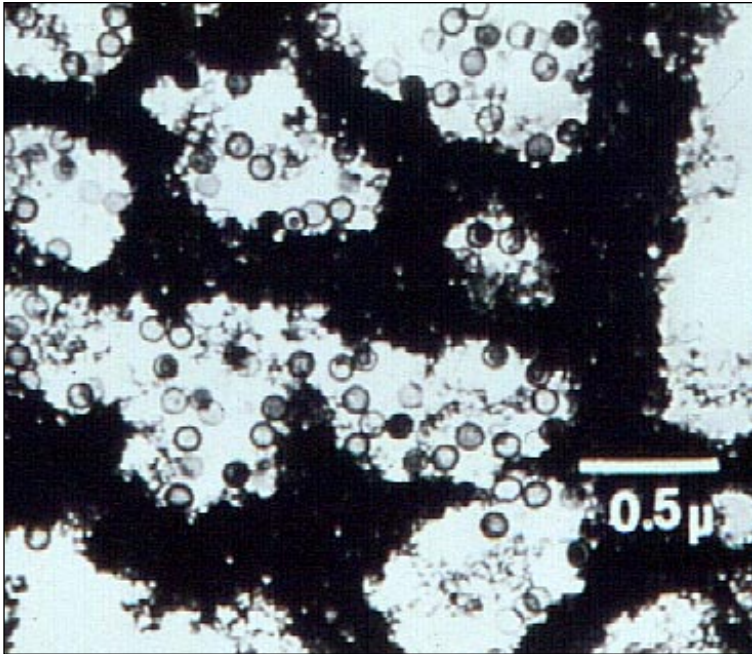


Figure 2: Structure of KSHV as visualized by electron microscopy<sup>6</sup>

Polymerase chain reaction (PCR) *in situ* hybridization studies have shown that KSHV is present in the endothelial cells lining the vascular spaces of the Kaposi's lesions as well as in the typical Kaposi's spindle cells, but not in the surrounding tissues.<sup>7</sup> These findings show that KSHV is present in the neoplastic cell types within these lesions.

Transmission studies using a cell line infected with KSHV and co-cultivated with a KSHV-negative recipient cell line were able to detect the presence of KSHV-positive cells in the resultant cell line, showing the ability of KSHV to infect other cell lines.<sup>3</sup>

## Oncogenic potential

There are two cellular homologues to proto-oncogenes in KSHV. The first is cyclin D (BCL-1) which is found in open reading frame (ORF) 72.<sup>8</sup> Human cyclin D is involved in cell cycle regulation, and dysregulation of cyclin D has been associated with a variety of tumours. Cyclin D expression has been observed in Kaposi's lesions as well as body cavity based lymphoma (BCBL) cell lines.<sup>9</sup> The other proto-oncogene homologue is found in ORF16 and bears a 59% amino acid identity to human BCL-2. In humans this is an anti-apoptotic gene disrupting programmed cell death and is associated with follicular lymphomas.<sup>10</sup>

## Kaposi's Sarcoma

First described in 1872 by Kaposi as an 'idiopathic pigmented sarcoma of the skin',<sup>11</sup> Kaposi's sarcoma is now classified according to four different epidemiological manifestations: classic, African endemic, iatrogenic and AIDS-associated (Table 1).<sup>12</sup> Classic Kaposi's sarcoma is commonly found in older men of Mediterranean origin and consists of skin lesions with little or no visceral involvement. Endemic Kaposi's sarcoma is found in southern equatorial Africa and can show systemic involvement with rapid progression. Iatrogenic immunosuppression-related Kaposi's sarcoma develops as a result of immunosuppressive medications in approximately 0.5% of organ transplant recipients, occurring 10–22 months post-transplantation.<sup>13</sup> Lesions usually remain localized and often resolve after decreasing the dose or discontinuation of immunosuppressive medication.<sup>14,15</sup> AIDS-associated Kaposi's sarcoma is the most aggressive form of the disease, with both cutaneous and visceral lesions.

Type	Risk group	Age at onset (years)	Male/female ratio
Classic	Patients with eastern European, Jewish and Mediterranean backgrounds	50–80	10–15:1
African endemic	Black African adults	30	3:1
Iatrogenic	Organ transplant recipients and patients with connective tissue or autoimmune diseases	20–60	2.3:1
AIDS-related	Homosexual men (95%) Other risk groups (5%)	18–65	106:1

Table 1: Epidemiology of Kaposi's sarcoma variants (modified from Zalla et al<sup>57</sup>)

### Clinical features in individuals with AIDS

Kaposi's sarcoma is a vascular tumour, which contains characteristic spindle cells. Clinically, disfiguring purplish/red vascular blotches appear on the skin. In the early stages these appear as bruise-like, flat lesions. Disease progression is rapid, however, with lesions becoming nodular and then ulcerated in advanced disease. Oral Kaposi's sarcoma is particularly common in individuals with AIDS and usually involves the hard palate, gingiva and tongue.<sup>16</sup> Twenty-two per cent of patients with AIDS-associated Kaposi's sarcoma present with oral lesions, while 45% present with oral lesions in addition to skin and/or visceral lesions.<sup>17</sup> Homosexual/ bisexual men with oral AIDS-associated Kaposi's sarcoma have a poor prognosis,<sup>16</sup> most having CD4 counts less than 200/mm<sup>3</sup> and an average survival time following diagnosis of 21 months (range 3–54 months).<sup>18</sup>

An increased risk of Kaposi's sarcoma in HIV-infected individuals with low CD4 counts has been confirmed by Gallant *et al* (1994), who found a 25% probability of Kaposi's sarcoma at 2 years in patients with initial CD4 counts less than 100/mm<sup>3</sup>, compared with 15% for patients with counts of 100/mm<sup>3</sup> or greater.<sup>19</sup> The 2-year risk of developing Kaposi's sarcoma in patients with CD4 counts of less than 250/mm<sup>3</sup> was 21%. This study found that Kaposi's sarcoma was an independent predictor of death; median survival after diagnosis of Kaposi's sarcoma was 408 days.<sup>19</sup>

## Association of an Aetiological Agent with the Development of Kaposi's Sarcoma

### Molecular biological data

Several analyses, using a PCR primer set amplifying a sequence of 233 base pairs from one of the DNA fragments (KS330<sub>233</sub>) originally isolated by Chang *et al* (1994),<sup>1</sup> have verified the specificity of the association of KSHV with Kaposi's sarcoma. Pooling the results of nine studies,<sup>20–28</sup> 211 (97%) out of a total of 224 Kaposi's sarcoma lesions examined were PCR positive. This includes all subtypes of Kaposi's sarcoma: classic, African endemic, iatrogenic and AIDS-associated. The results show that KSHV is universally found in Kaposi's lesions. Of 449 control tissues used in these studies only eight (2%) were positive for KSHV. Furthermore, KSHV appears to be localized to the

tumours themselves. Dupin and colleagues took biopsies of Kaposi's sarcoma lesions and found that with successive biopsies taken distally from the lesions they were less able to detect the KSHV DNA sequences.<sup>20</sup>

## *Epidemiological data*

Kaposi's sarcoma was uncommon prior to the outbreak of the AIDS epidemic but is now the most common AIDS-associated cancer of homosexual or bisexual men with AIDS;<sup>29,30</sup> the risk of developing Kaposi's sarcoma in these patients has been estimated at 20 000 times greater than that in the general population.<sup>31</sup> HIV-positive homosexual/bisexual men have about a 20 times greater risk of developing Kaposi's sarcoma than HIV-positive haemophiliacs.<sup>31</sup> Cohort studies demonstrate that up to one half of homosexual/bisexual men develop Kaposi's sarcoma over the course of their lifetime.<sup>30</sup> The risk is higher among women reporting sexual contact with bisexual men<sup>31</sup> and HIV-positive children born to mothers with high-risk factors for AIDS-associated Kaposi's sarcoma.<sup>32</sup> An increased risk of Kaposi's sarcoma has also been reported in homosexual men who are not infected with HIV.<sup>30</sup> Coincident with the African AIDS epidemic, Kaposi's sarcoma is now the most frequently reported tumour in several African cancer registries.<sup>33</sup> These observations suggest the involvement of a sexually transmitted agent in the development of Kaposi's sarcoma and that specific sexual practices among homosexual men may increase the probability of transmitting this virus.<sup>31,33,34</sup> Furthermore, a series of epidemiological and other studies have demonstrated that Kaposi's sarcoma is associated with behavioural and geographic risk factors most consistent with the tumour being caused by a sexually transmitted agent.<sup>31,34,36–41</sup>

To explore a possible sexual route of transmission of KSHV, Lin *et al* (1995) performed a retrospective blinded evaluation of semen collected from HIV-infected homosexual men in 1989 and 1990 to determine the presence of KSHV sequences in these samples.<sup>42</sup> Over 5 years of follow up, 43% of patients with KSHV detectable sequences in their semen and none of the KSHV-negative homosexual men developed Kaposi's sarcoma.<sup>42</sup>

Other studies have reported conflicting data on the incidence of KSHV sequences in the semen of both HIV-positive men and healthy donors. Lin *et al* (1995) found sequences in 91% of HIV-positive homosexual men and in 23% of specimens from healthy donors with a nested PCR assay.<sup>42</sup> An Italian study found KSHV sequences in the semen of 50–91% of a healthy donor population,<sup>43</sup> whereas another study could not detect the sequences in either the semen of HIV-positive or uninfected men.<sup>28</sup>

## *Evidence that infection precedes disease*

Among the epidemiological criteria for establishing a causal role for KSHV in Kaposi's sarcoma is the demonstration that infection precedes development of disease.

### *PCR data*

To examine the temporal relationship between KSHV infection and the development of Kaposi's sarcoma, Moore *et al* (1996) retrospectively examined peripheral blood mononuclear cell (PBMC) samples from patients with AIDS-associated Kaposi's sarcoma taken at a baseline examination prior to the development of Kaposi's sarcoma and immediately before or after sarcoma onset.<sup>44</sup> Patients with AIDS-associated Kaposi's sarcoma were significantly more likely to show evidence of KSHV

infection in PBMCs prior to the onset of Kaposi's sarcoma than the control groups who did not develop Kaposi's sarcoma (Table 2).<sup>44</sup>

Whitby *et al* (1995) found the sequences in 52% of PBMCs in patients with AIDS-associated Kaposi's sarcoma and in only 8% of individuals with AIDS without Kaposi's lesions; KSHV was not found in the PBMCs of 134 healthy blood donors.<sup>45</sup> Furthermore, KSHV DNA was detected in PBMCs of 11 HIV-infected individuals without Kaposi's sarcoma six of whom (55%) subsequently developed Kaposi's sarcoma compared with 12 out of 132 (9%) patients who were KSHV-negative.<sup>45</sup>

### Serological data

Immunoblot studies have identified two specific Kaposi's sarcoma-associated antigens, to which patients with Kaposi's lesions have reactive antibodies in their sera. Gao *et al* (1996) looked for the presence of these antibodies in the sera of HIV-positive individuals with lesions, HIV-positive homosexual men without lesions, HIV-positive haemophiliacs and healthy blood donors.<sup>46</sup> They found that 52% of HIV-positive individuals seroconverted before the clinical appearance of Kaposi's sarcoma, suggesting that there is a high-risk population for the development of the disease. The median duration of antibody seropositivity for KSHV-related latent nuclear antigens before the diagnosis of Kaposi's sarcoma was 33 months. For these patients, seropositivity rates increased linearly with time indicating that the rate of infection was constant. These results suggest that infection occurs prior to the development of the sarcoma, that the incubation time is quite short and that the risk of developing Kaposi's sarcoma once infected with KSHV is not highly dependent on the duration of infection.<sup>46</sup> Gao *et al* (1996) believe that the antigens are nuclear and have named them latency-associated nuclear antigens.<sup>46</sup> Healthy blood donors and individuals with AIDS without Kaposi's lesions are not usually seropositive for these antigens, again suggesting that KSHV is not routinely found in low-risk populations.

	Initial sample	Second sample
<b>◆ AIDS-KS (n=21)</b>		
Median time before or after AIDS-KS (months)	-13	+1
Median CD4 count (cells/mm <sup>3</sup> )	432	124
Number KSHV positive (%)	9 (43)	12 (57)
<b>◆ Homosexual/bisexual AIDS without KS (n=23)</b>		
Median time before AIDS diagnosis (months)	-55	-5
Median CD4 count (cells/mm <sup>3</sup> )	612	215
Number KSHV positive (%)	1 (4)	2 (9)
<b>◆ Haemophilic AIDS without KS (n=23)</b>		
Median CD4 count (cells/mm <sup>3</sup> )		344
Number KSHV positive (%)		2 (11)

AIDS-KS, AIDS-associated Kaposi's sarcoma. KS, Kaposi's sarcoma. Paired samples were not available for haemophilic control patients.

Table 2: Detection of KSHV DNA by PCR in AIDS-associated Kaposi's sarcoma, homosexual/ bisexual and haemophilic individuals with AIDS<sup>44</sup>

The retrospective analysis of PBMCs and results from the immunoblot studies suggest that KSHV infection is uncommon among individuals at low risk for Kaposi's sarcoma, in contrast to the results of recent PCR-based studies.<sup>42,43,47</sup> The observation that patients in whom the virus has been detected are at very high risk of developing the sarcoma, suggests that it is unlikely to be ubiquitous.<sup>45,46</sup>

## Association of Kaposi's Sarcoma-Associated Herpesvirus with Other Diseases

Unlike EBV, KSHV does not appear to be associated with a primary syndrome such as infectious mononucleosis, but has been detected in tissues from two related lymphoproliferative disorders: body cavity based lymphoma (BCBL) and multicentric Castleman's disease.

### BCBLs

Examination of a variety of AIDS and non-AIDS lymphomas has identified KSHV sequences in a unique subgroup of AIDS-related B-cell lymphomas, BCBLs, but not in any other lymphoid neoplasms; these lymphomas also contained EBV.<sup>47-49</sup> Out of a total of 193 lymphomas from 42 individuals with AIDS and 151 individuals without HIV infection, sequences were identified in eight lymphomas in HIV-positive individuals.<sup>48</sup> All eight were BCBLs, also known as primary effusion lymphomas.<sup>48</sup> KSHV sequences were not found in the other 185 lymphomas.<sup>48</sup> The high degree of conservation between the KSHV sequences in Kaposi's sarcoma in the eight BCBLs suggests the presence of the same agent in both lesions. The KSHV virus loads were higher in the BCBLs than in the Kaposi's sarcoma lesions, suggesting that the lymphomas are an independent pathological process from Kaposi's sarcoma.<sup>48</sup> The BCBLs are clinically and biologically unique and found predominantly in patients with advanced AIDS.<sup>50</sup> They have a tropism for pleural, pericardial and peritoneal cavities and present as malignant effusions and not solid masses.

The KSHV infected BCBL tumours examined to date are of monoclonal B cell origin without c-myc gene rearrangement.<sup>51</sup> Recently, BCBLs that are not KSHV-infected have been identified. In contrast to the KSHV-infected BCBLs, the KSHV-negative BCBLs have c-myc rearrangements suggesting that such rearrangement and KSHV infection may be alternate pathways to the same tumour phenotype.<sup>51</sup>

### Castleman's disease

Castleman's disease or giant lymph node hyperplasia is a rare disorder that is characterized by enlarged hyperplastic lymph nodes. There are two subtypes – a localized form and a multicentric form – which have very different clinical presentations. The localized form usually affects young adults and presents as a mediastinal or retroperitoneal mass; excision is generally curative. The multicentric form, with systemic manifestations of the disease such as fever, anaemia, hypergammaglobulinaemia and weight loss, has a poor prognosis and has been associated with KSHV. Soulier *et al* (1995) examined 31 patients with multicentric Castleman's disease for the presence of KSHV sequences.<sup>52</sup> The sequences were detected in 14 out of 14 cases of HIV-associated multicentric Castleman's disease,

including five cases without detectable Kaposi's sarcoma, and in seven out of 17 cases in HIV-negative individuals.<sup>52</sup>

Additional studies are required to determine whether multicentric Castleman's disease in individuals with AIDS and HIV-negative individuals are distinct pathophysiological processes and to determine the role of KSHV in these disorders.<sup>51</sup>

## Treatment of Kaposi's Sarcoma

There is currently no evidence to suggest that antiviral therapy is effective in the treatment of Kaposi's sarcoma. Kaposi's sarcoma lesions can regress in transplant recipients after the immunosuppressive therapy is discontinued,<sup>14</sup> and regression and even complete remission have been described in severely immunocompromised patients including those with AIDS.<sup>54</sup> Regression of Kaposi's sarcoma lesions has also been reported in three out of five patients treated with foscarnet.<sup>55</sup> Another small study has associated foscarnet with a decreased risk, but showed no benefit of aciclovir or ganciclovir.<sup>56</sup> Both studies were retrospective analyses.

Current treatment regimens include local destructive measures, such as cryotherapy, surgical excision and radiation therapy, and systemic regimens with chemotherapeutic agents such as interferon and the cytotoxic vinblastine.<sup>57</sup> The choice of treatment is based on the extent of the disease, degree of immunosuppression and concurrent medical problems.<sup>57</sup>

## Summary and Management Recommendations

### *Evidence for a new herpesvirus, KSHV*

Isolation of viral DNA sequences from an AIDS-associated Kaposi's sarcoma lesion using representational difference analysis (RDA) has identified a new herpesvirus, KSHV. Virologic characterization studies and phylogenetic analysis, have assigned KSHV to the gammaherpesvirus subfamily.

### *Epidemiological studies of Kaposi's sarcoma*

Epidemiological studies have long suggested that Kaposi's sarcoma is caused by a sexually transmitted agent and the coincident increase in the disease with AIDS has reinforced this notion. Unlike other AIDS-related malignancies, AIDS-associated Kaposi's sarcoma primarily occurs in specific HIV-risk groups, shows geographical clustering and may be associated with specific sexual behaviours. Healthy blood donors and individuals with AIDS without Kaposi's lesions are not usually seropositive, suggesting that KSHV is not an ubiquitous infection among low-risk populations.

### *Causal role of KSHV in Kaposi's sarcoma*

The RDA-generated data have facilitated investigations on the epidemiology of Kaposi's sarcoma and the role of an infectious agent in its pathogenesis. In addition to AIDS-associated Kaposi's lesions, KSHV has been detected in all clinical/epidemiological subtypes of the disease, including classic, African endemic and iatrogenic Kaposi's sarcoma. KSHV has also been detected in two related lymphoproliferative disorders: BCBL and multicentric Castleman's disease, suggesting the virus has a generalized pleiotropic transforming effect. In contrast, viral KSHV DNA is generally not found in non-Kaposi's solid tissues supporting the belief that KSHV is aetiologically related to the sarcoma.

Further evidence for a role of KSHV in Kaposi's sarcoma has come from serological studies and the report of higher incidences of KSHV DNA sequences in peripheral blood mononuclear cells of patients with AIDS-associated Kaposi's lesions than in individuals with AIDS without Kaposi's lesions. Infection has been demonstrated to precede development of disease, establishing a causal role of KSHV in Kaposi's sarcoma.

### *Transmission of KSHV*

A sexual route of transmission of the virus is likely in view of the very high risks of developing Kaposi's sarcoma in homosexual/bisexual men infected with HIV and in HIV-negative homosexual men and HIV-positive women with whom they have sexual relationships. However, given the detection of KSHV in all subtypes of Kaposi's sarcoma and the frequency of non-AIDS associated forms in Mediterranean countries, where the disease predominantly affects older men and displays no evidence for sexual transmission, there must also be an alternative means of transmission. The mode of transmission of the virus therefore needs to be clarified and may then explain the varying epidemiology of the virus worldwide.

### *Future issues*

More data are required on the epidemiology of Kaposi's sarcoma in different areas of the world particularly in view of the fact that despite an AIDS epidemic in Thailand, Kaposi's sarcoma is extremely rare and most of the cases are believed to have been imported.

To improve the epidemiological data and to identify patients at risk of developing disease the availability of even more sensitive serological tests are required as current assays are only able to detect an antibody response in approximately 80% of Kaposi's sarcoma patients. More data are also needed on the risks of KSHV transmission to sexual partners so that decisions can be made on whether messages about the risks of transmission of KSHV should be included in advice about safer sexual practices.

## Herpesviruses – Co-Factors in HIV Progression?

With declining immune function in individuals with AIDS there may come a time when herpesviruses become involved in the deterioration of the patient. Furthermore, they are likely to assume even greater significance as improvements in therapy enable patients to survive longer, not just by causing disease in their own right but also by potentially interacting with HIV.

The role of viral co-factors in the pathogenesis of HIV is controversial and continues to be debated. Since the *Management Strategies* publication, *Herpesviruses and HIV Infection – Co-Factors and Opportunistic Infections*,<sup>1</sup> additional *in vitro* and *in vivo* data have accumulated to support herpesvirus involvement and are presented in this update. Readers are referred to the previous publication for more detailed background information and for schematic representations of the proposed mechanisms for interactions between herpesviruses and HIV that are discussed below.<sup>1</sup>

### The Co-Factor Hypothesis

Although the average length of time from seroconversion to the development of AIDS is 10 years, there is wide variation among individuals in the length of this period, ranging from 2 years to 20 years.

A number of factors may influence the duration of the incubation period (Table 1),<sup>2</sup> including the possibility that herpesviruses may act as co-factors in HIV disease progression. A co-factor may be defined as an infectious agent which interacts at the molecular or cellular level to promote HIV pathogenicity.

- Initial inoculum of HIV
- Subsequent inocula of HIV
- Rates of HIV genetic change
- Genetic predisposition to control HIV replication or trigger immunopathological responses
- Immune function (dysfunction)
- Age
- Infectious co-factors

Table 1: Potential factors affecting AIDS incubation period

Potential infectious co-factors in HIV infection can be investigated *in vitro* or *in vivo* in human tissues, human population studies or in clinical trials. However, there are inherent difficulties in determining if any one co-factor is involved, as the study must also control for all of the remaining parameters.

### Levels of HIV – Co-Factor Interaction

#### In vitro data

To interact with HIV, the co-factor virus must either be found in the same cell or in a neighbouring cell interacting via a bystander effect. Several mechanisms have been proposed by which this interaction may occur, some of which have now

been substantiated by *in vitro* evidence (Table 2).<sup>3</sup>

#### Single cell interactions

Single cell mechanisms for the action of viral co-factors propose that the co-factor virus and HIV infect the same cell. For these models to apply, the course of infection of cells

Virus	Transactivation	Cytokine release	Receptor activation	Antigen presentation	Pseudotype formation
HSV	✓	✗	–	–	✓
VZV	–	✗	–	–	–
EBV	✓	✓	–	–	–
CMV	✓	✓	✓	✓	✓
HHV-6	✓	✗	✓	–	–
HHV-7	–	–	✗	–	–
KSHV	–	–	–	–	–

✓ supporting data. ✗ interaction not found in vitro. – indicates no data.  
 HSV, herpes simplex virus. VZV, varicella zoster virus. EBV, Epstein-Barr virus. CMV, cytomegalovirus. HHV-6 and 7, human herpesvirus types 6 and 7.  
 KSHV, Kaposi's sarcoma-associated herpesvirus.

Table 2: In vitro evidence for or against herpesviruses as co-factors

with the co-factor virus must be trophic but less rapidly lytic in target cells, otherwise the cell would be destroyed before the HIV genome could be replicated. For this reason, herpes simplex virus (HSV), a virus which causes rapid lysis of infected cells, is less likely to be a co-factor than more slowly replicating herpesviruses.

**Transactivation:** According to the transactivation model, the co-factor virus produces a protein which interacts directly or indirectly with the promoter of HIV to stimulate HIV replication. A number of herpesviruses have transactivating proteins which up-regulate HIV expression *in vitro*.<sup>4-6</sup> Several human herpesvirus type 6 (HHV-6) transactivator genes have been identified and the HIV *tat* gene has been shown to enhance replication of HHV-6.<sup>7</sup> As HHV-6 is cytopathic, its activation by HIV may also accelerate the depletion of CD4 cells in infected individuals.

**CD4 up-regulation:** The main receptor for HIV types 1 and 2 on T- and B-lymphocytes, monocytes and macrophages is the CD4 antigen although co-receptors such as Fusin on T-cells and CC CKR5 on macrophages are also required.<sup>8-10</sup> By switching on a cell's CD4 gene and rendering it CD4-positive, a herpesvirus may increase the number of cells susceptible to HIV infection. Lusso *et al* (1991) have demonstrated that infection with HHV-6 dramatically up-regulates the expression of CD4.<sup>11</sup> Moreover, HHV-6 induces *de novo* expression of CD4 messenger RNA and protein in normal mature CD8 lymphocytes rendering them susceptible to infection with HIV.<sup>11</sup>

There is also the theoretical possibility that some herpesviruses may have a negative co-factor effect on HIV. CD4 is an essential component of the cellular membrane receptor for human herpesvirus type 7 (HHV-7) and a marked reciprocal interference between HHV-7 and HIV has been observed.<sup>12</sup> It will be interesting to investigate whether active infection with HHV-7 influences the natural history of HIV infection *in vivo*, either as an effective natural inhibitor of HIV infection or by enhancing its cytopathic effects.

**Alternative receptor:** In addition to using CD4 and co-receptors, HIV can also infect monocytes and macrophages by means of receptors for the Fc portion of immunoglobulins or complement receptors. HSV and cytomegalovirus (CMV) have been shown to induce expression of these receptors, thereby providing HIV with an alternative receptor to CD4. Furthermore, Fc receptors induced by CMV have been shown to allow HIV to infect fibroblasts otherwise not permissive to HIV infection.<sup>13</sup>

**Herpesvirus–HIV pseudotype formation:** If a cell were co-infected with both viruses, it is possible that on exiting the plasma membrane, the HIV RNA may acquire the surface glycoproteins of the herpesvirus, endowing it with the ability to infect cells lacking a CD4 receptor.<sup>14</sup>

### Cell-to-cell interactions

Other models for the interaction between HIV and a co-factor virus propose that the viruses infect different cells.

**Cytokine release:** Infection with the co-factor virus may trigger the release of cytokines from the infected bystander cell. Through a process of signal transduction these may activate similar transcription factors in the HIV-infected cell (e.g. NF-Kappa B) which would activate the latent HIV DNA.

**Antigen presentation:** Infection with the co-factor virus may result in the release of viral proteins from the infected cell. Again through a process of signal transduction, these proteins may then be recognized by the antigen receptor of a herpesvirus-primed T-memory cell which is harbouring HIV.

### In vivo data

Although all of the herpesviruses except VZV have some *in vitro* evidence to support a role as a co-factor, CMV, HSV and HHV-6 are the most consistently implicated and are also supported by several pieces of *in vivo* evidence.

Careful selection of patients is necessary for clinical studies to investigate any direct effect of a putative viral co-factor. Results from one such study, which controlled for confounding factors, have suggested that CMV may act as a co-factor in the development of AIDS.<sup>3</sup>

To determine whether CMV seropositivity in haemophiliacs at the time they contracted HIV affected the time to developing AIDS, a cohort of approximately 100 patients were studied. Of these, 13% who were CMV-seronegative developed AIDS compared with 41% of those who were CMV-seropositive. The Kaplan-Meier plot of time from HIV seroconversion to the development of AIDS shows that the probability of progression to AIDS was greater in patients who were seropositive for CMV (Figure 1).<sup>3</sup> Such an effect was not seen in a second group of haemophiliacs.<sup>15</sup> In prolonged follow up of the original study, CMV-seropositivity also increased the risk of death and was associated with a more rapidly declining CD4 count.<sup>16</sup>

An association of CMV with HIV disease progression has also been demonstrated in a study of 234 HIV-positive men who were prepared to give semen samples every 3–6 months. A total of 164 gave more than four samples and were divided into three groups: consistently CMV-negative, intermittently CMV-positive and persistently CMV-positive. The latter group was associated with a more rapid progression to AIDS even after controlling for differences in CD4 count (Table 3).<sup>17</sup> A more rapid progression to AIDS was also associated with the presence of multiple CMV strains.

HSV and HIV interaction has been demonstrated by Heng *et al* (1994) who found that in tissues co-infected with HSV-1, HIV was observed to infect keratinocytes, which because they lack the CD4 molecule are normally incapable of being infected by HIV.<sup>18</sup>

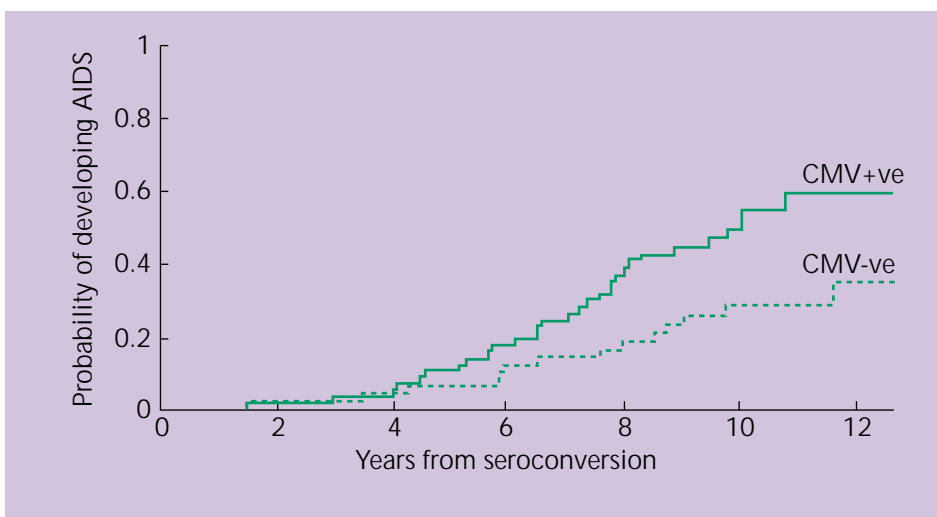


Figure 1: Kaplan-Meier plot of time from HIV seroconversion to the development of AIDS for CMV-seropositive and -seronegative patients<sup>16</sup>

Group	No. of patients	Median CD4 (cells/mm <sup>3</sup> )	No. of individuals with AIDS	Relative hazard
Persistently negative	58	492	3	1
Intermittently positive	54	501	6	2.9
Persistently positive	52	348	14	4.0

Table 3: HIV-positive individuals persistently positive for CMV in their semen are at greatest risk of developing AIDS<sup>17</sup>

Heng *et al* took skin biopsies of non-genital skin from six patients with AIDS and HSV infection who had very low CD4 counts, six matched HIV-negative individuals who had HSV infection and 10 HIV-positive individuals who had dermatitis not caused by HSV. They counted the average number of virions per cell and found that where both viruses were present there were many more virions (Table 4). Although a number of other viruses have been reported to enhance HIV transcription *in vitro*, this is the first *in vivo* report of reciprocal enhancement of viral replication associated with co-infection of keratinocytes and macrophages by HIV and HSV-1 in individuals with AIDS and non-genital HSV lesions. Some of the virions in the co-infected cells were larger and morphologically atypical which suggests that they may be pseudotypes.<sup>18</sup>

Cell type	No. of cells examined	Average no. of virions per cell		
		HIV-/HSV <sup>+</sup>	HIV <sup>+</sup> /HSV <sup>-</sup>	HIV <sup>+</sup> /HSV <sup>+</sup>
Keratinocyte	560	31	0	736
Macrophage	750	0	12	866
Lymphocyte	1260	0	2	458

Table 4: Influence of HSV and HIV co-infection on cell productivity<sup>18</sup>

Another study implicating HSV in the progression of HIV has shown that HSV recurrences increase the viral burden of HIV as measured by plasma load of HIV RNA. So that other explanations could not be used for alterations in HIV virus load, entry criteria were strict:

patients in whom HSV was isolated had received aciclovir, were clinically stable 3 months before and 3 months after testing and had been compliant with their antiretroviral therapy over the previous 6 months. Eight patients met the requirements and serum samples were collected 30–45 days before or after the episode of genital HSV. The investigators found that during acute infection, HIV RNA increased, and then decreased afterwards indicating that HSV can up-regulate plasma HIV RNA.<sup>19</sup>

Recently, Schacker *et al* (1996) reported a study to evaluate the frequency of HIV isolation in herpetic lesions of HSV-2 seropositive, HIV-positive men during the course of 1–5 HSV-2 recurrences.<sup>20</sup> HIV PCR analysis and HSV culture was performed on the swabs from 12 HIV-positive men with genital HSV. These individuals experienced a total of 26 episodes and, for each individual, the lesions were of *normal* size. HIV RNA was detected in 25 of the 26 episodes of genital HSV and HIV RNA was detected in genital swabs on 66% of the days. The HIV RNA that was isolated was virion RNA and appeared replication competent. HIV RNA was not detected in genital swabs in the absence of genital HSV lesions.<sup>20</sup> These data suggest that HIV production is increased in the presence of HSV and that genital HSV lesions may facilitate the transmission of HIV.

## Autopsy data

If a herpesvirus is to interact with HIV, both viruses must be found within the same organs of the body. It is of interest to note that autopsies frequently show that patients with AIDS die with herpesviruses in major organs. In a prospective study of 47 autopsies on individuals who had AIDS, 66% had active infection with at least one herpesvirus. CMV, HSV and HHV-6 are the most frequently detected. HHV-6 has been found in 85% of tissues from individuals with AIDS compared with only 53% of controls and is found in HIV-infected T-cells.<sup>21–23</sup>

Site	Patients with HIV/CMV co-infection (%)
Lung	69
Heart	58
Brain	57
Adrenal	54
Saliva	50
Spleen	50
Liver	47
Kidney	21

Table 5: Location and frequency of HIV/CMV co-infection<sup>24</sup>

Examination of 116 organs from 16 patients showed that 52% had both CMV and HIV detectable (Table 5).<sup>24</sup> Evidence for HIV/CMV dual infection of individual cells has been demonstrated by a number of investigators since it was first detected by Nelson *et al*.<sup>25–28</sup>

## Clinical trial data

From available evidence it is clear that people with AIDS die with herpesvirus infections but do they die from them? Several trials have shown that inhibiting herpesviruses appears to be associated with improved survival in individuals

with AIDS.<sup>29–33</sup> Although the mechanisms behind the observed survival benefit are not fully understood, some researchers have suggested that it may be due to the effects of aciclovir on certain herpesviruses which otherwise would act as co-factors.

Support for this hypothesis comes from an analysis of 786 HIV-positive homosexual or bisexual men from the Multicentre AIDS Cohort Study (MACS), who had begun

zidovudine therapy before a clinical diagnosis of AIDS.<sup>34</sup> At each visit, individuals were asked whether they had used any medication for health reasons not related to AIDS and whether they had taken any to help fight AIDS or HIV. The results showed that the use of aciclovir for any indication was not associated with an effect on progression to AIDS but was associated with a 26% decrease in the risk of death ( $P=0.07$ ). The use of aciclovir for HIV infection was again not associated with an effect on progression to AIDS, but was associated with a 36% decrease in the risk of death ( $P=0.01$ ). Further investigation of these findings, examining how constantly aciclovir was taken and at what dose, found no apparent dose effect on survival but that longer, uninterrupted use of aciclovir was associated with a survival benefit.<sup>34</sup>

## Summary and Management Recommendations

### *Evidence for HIV – co-factor interaction*

The wide range of times between primary infection with HIV and the development of an AIDS-defining illness when compared with those for other viral infections suggests that factors other than HIV may be involved in the pathogenesis of AIDS. The role of herpesviruses as opportunistic pathogens in HIV infection is well documented, but their role as co-factors in the pathogenesis of HIV infection can only be demonstrated clearly if other confounding factors are controlled for or excluded, i.e. inoculum of HIV, age, genetic predisposition, strain of HIV and other diseases including opportunistic herpesvirus infections. Such trials have generally not been conducted.

That herpesviruses may act as co-factors in the progression of HIV is suggested by several lines of *in vitro* and *in vivo* evidence including the recent data on KSHV summarized in Chapter 1. In addition, controlled clinical trials of aciclovir and zidovudine co-therapy in patients with AIDS show a significant survival benefit, but no effect on CMV disease, further implicating herpesviruses in HIV disease progression.

Although not originally designed to assess the effect of aciclovir on survival, two of three trials have shown a survival advantage in patients who had received long-term administration of aciclovir. To ascertain fully the value of this approach, placebo-controlled trials of continuous aciclovir (and other herpes agents) should be conducted in patients receiving highly active antiretroviral therapy. Patients with CD4 counts  $<200$  cells/mm<sup>3</sup> and unresponsive to available antiretroviral therapy, or with increasing HIV viral load should be made aware of all the data on aciclovir.

The involvement of herpesviruses in HIV progression is plausible although current data are mostly *in vitro*. *In vivo* data for the co-factor theory are harder to obtain but are accumulating and further studies are now warranted. More data are required on which herpesviruses are important as co-factors in HIV. Current data strongly implicate HSV, CMV and HHV-6 as the most important co-factor viruses. Future trials with novel, potent herpes drugs are therefore strongly indicated.

## Treating Cytomegalovirus Retinitis in HIV-Positive Individuals

With the increasing efficacy of antiretroviral therapies and prophylaxis or treatment for other opportunistic diseases, more individuals are reaching the later stages of HIV disease at which time reactivation of latent cytomegalovirus (CMV) tends to occur. Management of CMV infection in individuals with HIV infection is therefore becoming increasingly important.

### Natural History

CMV retinitis is the most common cause of visual loss in individuals with AIDS, with 15–40% of patients affected.<sup>1–3</sup> CMV retinitis usually occurs late in the course of AIDS. One retrospective study found that the median time between the diagnosis of AIDS and the development of CMV retinitis was 9 months.<sup>4</sup> Prospective epidemiological studies have estimated the risk of CMV retinitis in patients with CD4 counts less than 100 cells/mm<sup>3</sup> at approximately 10% per year and in those with CD4 counts less than 50 cells/mm<sup>3</sup> at approximately 20% per year.<sup>3,5</sup> Patients with CD4 counts above 200 cells/mm<sup>3</sup> have rarely developed CMV retinitis;<sup>6</sup> however, this could change in the future with widespread use of highly active antiretroviral regimens that dramatically change absolute CD4 counts.

Retinal infection with CMV usually presents clinically as a slowly progressive, necrotizing retinitis (Figure 1 a, b).<sup>2,7,8</sup> Affected areas will become atrophic and scarred, and as the eye is part of the central nervous system and does not have regenerative



Figure 1a: Photo of CMV retinitis in untreated patient



Figure 1b: CMV retinitis in untreated patient 5 weeks later, showing progression

power, prognosis is poor.<sup>2</sup> Untreated CMV retinitis may lead to blindness over several months as a result of progressive or new retinal lesions, optic atrophy and/or retinal detachment.<sup>7,8</sup> Jabs *et al* (1989) found that 60% of patients presenting with unilateral disease developed bilateral lesions without treatment.<sup>8</sup> The median progression rate of untreated, pre-existing lesions in one study was 24.0  $\mu\text{m}/\text{day}$  (range 0–164  $\mu\text{m}/\text{day}$ ).<sup>9</sup> Recurrences of disease occur rapidly after cessation of therapy (on average 28 days).<sup>8</sup>

## Standard Therapy

There are currently two antiviral drugs which have been approved by regulatory agencies throughout the world for the treatment of CMV retinitis – ganciclovir and foscarnet.<sup>10,11</sup>

### Ganciclovir

Studies have shown that 80–100% of patients with CMV retinitis show an initial improvement during ganciclovir therapy. A prospective, randomized controlled trial of induction therapy with intravenous ganciclovir 5 mg/kg every 12 hours for 14–21 days followed by maintenance ganciclovir 5 mg/kg once daily demonstrated a statistically significant increase in the median time to progression compared with patients whose treatment was deferred until they had evidence of progression.<sup>12</sup> Other studies have used less frequent intravenous regimens (10 mg/kg three times/week, 10 mg/kg three to seven days/week or 6 mg/kg 5 days/week),<sup>13</sup> with similar success, increasing the frequency of administration if early relapse occurs.

The most common serious adverse effect of ganciclovir is neutropenia. Among individuals with AIDS, 16% have been reported to have dose-limiting neutropenia (defined as an absolute neutrophil count  $<500$  cells/mm<sup>3</sup>) and 5% to have dose-limiting thrombocytopenia (platelet count  $<20\,000$ /mm<sup>3</sup>).<sup>14</sup> The risk of severe neutropenia is increased in patients taking both ganciclovir and zidovudine.<sup>15</sup>

### Foscarnet

Foscarnet has been shown to improve CMV retinitis in 87–97% of patients. Induction therapy is achieved with a continuous infusion of 90 or 100 mg/kg every 12 hours and maintenance therapy with an infusion of 90 or 120 mg/kg every 24 hours. The most common serious adverse effect of foscarnet is a dose-limiting nephrotoxicity, occurring in 10–23% of patients treated and manifesting as an increased serum creatinine level.<sup>16,17</sup> In order to limit foscarnet-induced nephrotoxicity, patients must be kept well hydrated.

### Comparative studies

There has been one large randomized trial of ganciclovir versus foscarnet.<sup>17</sup> In this multicentre trial, 234 patients with newly diagnosed CMV retinitis initiated therapy with either ganciclovir or foscarnet. There was no difference between the groups in the rate of retinitis progression, but survival was significantly longer in the foscarnet-treated group (median 12.6 months versus 8.5 months for the ganciclovir group). However, dose-limiting toxicity occurred in 20% of foscarnet- versus 1% of ganciclovir-treated patients. A subsequent clinical trial of combination therapy versus monotherapy failed to validate this observation.

### Treatment of relapsed retinitis

Retinitis progression usually responds to a re-induction course of the same therapy used initially, but the interval between relapses progressively shortens.<sup>18</sup> Progression is most likely to occur in patients who must interrupt therapy due to drug toxicity, but may also occur in patients taking full dosages as a result of progressive immune failure or drug resistance.

### Combination therapy

Synergistic antiviral activity of ganciclovir and foscarnet has been demonstrated *in vitro* and *in vivo*<sup>19</sup> and clinical studies of combination therapy have shown an

increased time to retinitis progression.<sup>20-22</sup> Combination therapy with ganciclovir and foscarnet at standard doses should be considered in patients with a relapse of retinitis or who show progressive retinitis while undergoing sustained monotherapy. The two drug preparations are incompatible for simultaneous administration, however, and so sequential infusions are necessary.<sup>20</sup> The disadvantages of combination therapy are a prolonged daily infusion time and the additive toxicity of the two different drugs.

## New Options for Maintenance Therapy

Following treatment of retinitis, maintenance therapy is necessary. Jacobson *et al* (1988) found that relapse of CMV retinitis occurred on average 16 days after discontinuation of therapy.<sup>23</sup> However, even in patients receiving chronic maintenance, progression (relapse) of disease will eventually occur and so regular ophthalmological follow-up is essential to monitor the progress of lesions.

Current standard ganciclovir or foscarnet regimens necessitate chronic daily intravenous drug administration, therefore the development of new options for CMV therapy has focused on strategies to avoid this.

### Oral ganciclovir

Oral ganciclovir is now approved in several countries for daily suppressive therapy to control CMV disease progression. Three randomized, open-label studies have compared maintenance treatment with oral ganciclovir (500 mg every 4 hours or 1000 mg every 8 hours) versus intravenous ganciclovir (5 mg/kg once daily) in patients with CMV retinitis following stabilization with intravenous ganciclovir (Table 1).<sup>24-27</sup>

	Drew <i>et al</i> <sup>24</sup>		Squires <i>et al</i> <sup>26</sup>		European and Australian <sup>27</sup>	
	IV	Oral	IV	Oral	IV	Oral
◆ Number of patients	121		225		159	
◆ Median CD4 count (cells/mm <sup>3</sup> )	9.5		7		10	
◆ Mean time to first progression of CMV retinitis (days)	62	57	65	54	62	51
◆ Patients with deterioration of visual acuity (%)	25	20	16	19	11	16

Table 1: Effectiveness of maintenance therapy using oral or intravenous (IV) ganciclovir in delaying first progression of cytomegalovirus retinitis (Adapted from Ward-Able *et al*<sup>28</sup>)

In all three trials there were trends, which did not reach statistical significance, toward better control of retinitis with intravenous rather than with oral ganciclovir. In one study there was also a trend toward more frequent extension into Zone 1 of the retina (within 3000 µm of the fovea or within 1500 µm of the optic nerve head, an area critical for vision) in patients who received oral ganciclovir and a trend towards patients developing disease in the contralateral eye.<sup>24</sup> The side-effects of intravenous ganciclovir were still seen with the oral formulation but tended to be both less severe and frequent.

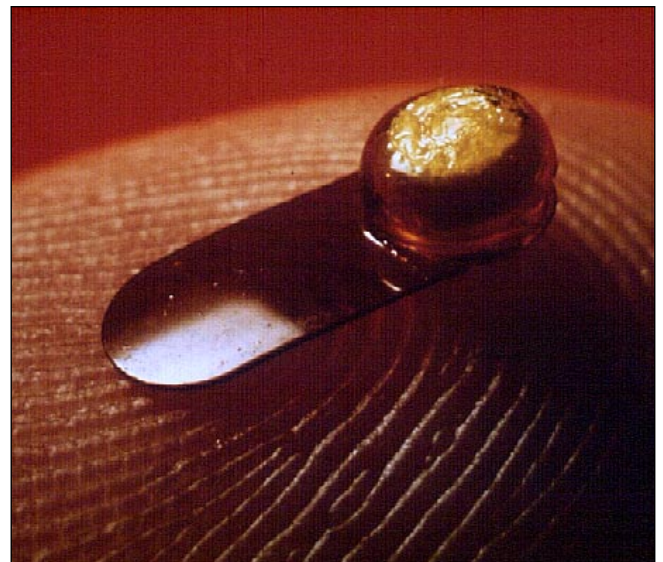
From available evidence, it appears that oral ganciclovir probably does not control progression of retinitis as well as intravenous ganciclovir because of its limited oral

bioavailability. In addition, median follow-up times of these studies were short so the long-term outcome is unknown. Furthermore, oral ganciclovir is less effective in patients who have received more than 100 days of prior intravenous ganciclovir treatment or who have had any prior retinitis progression.<sup>25</sup> Oral ganciclovir would therefore not be the drug of choice for a patient in whom a lesion was immediately sight threatening (i.e. in Zone 1) or in a patient with extensive peripheral disease for whom retinal detachment is a real risk.

Although the efficacy of oral ganciclovir may be slightly less than the intravenous formulation, it still appears to delay the time to progression of CMV retinitis compared with no treatment and offers clear advantages to the patient in terms of convenience, e.g. less severe side-effects; no daily intravenous infusions, whether in the clinic or in the home; and no requirement for a permanent indwelling catheter with associated risk of infection.

### *Ganciclovir intraocular implant*

A sustained-release intraocular implant has been developed which consists of a semi-permeable polymer containing a 6 mg ganciclovir pellet (Figure 2). Implanted under the sclera in the far anterior of the retina, where it does not interfere with vision, the device releases ganciclovir at a continuous rate of 1–2  $\mu\text{g}/\text{hour}$ , bathing the vitreous in levels of ganciclovir that are considerably higher than those that can be achieved with intravenous administration.<sup>29</sup> Visual distortion occurs for 2–4 weeks following the procedure. The life of the implant is approximately 6–8 months at which time patients must undergo further surgery for a replacement implant.



*Figure 2: Intraocular implant*

The implant has been evaluated in two randomized controlled trials, the largest of which involved 173 evaluable patients and compared a 1  $\mu\text{g}/\text{hour}$  release rate implant or a 2  $\mu\text{g}/\text{hour}$  implant with standard intravenous ganciclovir therapy.<sup>30</sup> The other study was smaller and looked at immediate versus deferred treatment in 26 patients (30 eyes) with newly diagnosed peripheral retinitis (outside of Zone 1). Patients were randomized to have the implant placed immediately (1  $\mu\text{g}/\text{hour}$  release rate) or have therapy deferred until disease progression was observed.<sup>31</sup>

The results of the two studies show that the median time to progression (by involved eye) for treated eyes with retinitis at baseline was significantly longer for the implant, 216 days<sup>29</sup> and 226 days<sup>29</sup> than for the intravenous ganciclovir group, 104 days. The deferred treatment time to progression was 15 days.<sup>31</sup>

Although the efficacy of this implant is the best reported to date for any CMV retinitis treatment and avoids the morbidity associated with central venous access, the surgical procedure can be associated with serious ocular adverse events. Results are consistent in that approximately 10% of patients in each study had an adverse visual outcome that was associated with the surgical procedure, either an early retinal detachment (which made up most of the adverse outcomes) or a rare case (0.5%) of bacterial ophthalmitis or a serious retinal haemorrhage.<sup>30,31</sup> Patients who are implanted in one

eye and are not receiving systemic therapy are at very high risk of developing retinitis in the untreated, fellow eye (40–50% of patients at 6 months). They are also at risk of developing extraocular disease (15–31% of patients). Patients should be informed of the relative risks and benefits of the intraocular implant.

The USA *Food and Drug Administration* (FDA) has approved the intraocular ganciclovir implant for the treatment of CMV retinitis in individuals with AIDS. However, its use in patients with peripheral disease may be counter-productive. Retinal detachment is part of the natural history of CMV retinitis and will occur in about one third of patients during their life. It seems to occur most frequently in patients who have large peripheral lesions as this is where the retina is attached. The implant is likely to offer most benefit to patients with useful functional vision and Zone 1 lesions as any progression is likely to cause an enlarged visual loss, either a visual field deficit or serious visual impairment. Although trial data are not available, it is suggested that because of the risk of extraocular disease or the development of retinitis in the untreated, fellow eye, the implant should not be advised without some form of concurrent systemic therapy, possibly oral ganciclovir.

### *Intravitreal therapy*

The toxicity, cost and inconvenience of intravenous therapy of CMV retinitis has prompted the use of local intravitreal therapy in some cases. Henry *et al* (1987) demonstrated that a single 200 µg intravitreal injection of ganciclovir achieved vitreous levels of the drug above the 50% inhibitory concentration of most CMV strains for a 62-hour period.<sup>32</sup> Subsequently, several studies using intravitreal injections of 200–400 µg of ganciclovir<sup>33</sup> or 2400 µg of foscarnet<sup>34</sup> have achieved results comparable to those with intravenous therapy. Cidofovir (HPMPC) is a potent, long-acting anti-CMV agent that has shown promise as an intravitreal therapy and may be given monthly.<sup>35</sup>

The risks of repeated intravitreal injections include bacterial endophthalmitis, retinal detachment, vitreous haemorrhage, changes in intraocular pressure and possible retinal drug toxicity. Intravitreal injections are useful for patients who are temporarily unable to tolerate intravenous therapy because of drug toxicity.

An important limitation of both the ganciclovir intraocular device and intravitreal injections is that both approaches treat only one eye. Some of the benefits of intravenous therapy, including reduction in the development of bilateral disease, involvement of other organs and possibly enhanced survival are therefore presumably lost with local therapy. Some form of systemic therapy, therefore, should be considered in addition to intravitreal injections, to protect the contralateral eye and other anatomical sites.

### *Cidofovir*

Cidofovir is the most potent CMV drug *in vitro* that has come into clinical development to date and is already available in the USA for patients who have had difficulty tolerating or have failed therapy with standard ganciclovir or foscarnet.

In a Phase II/III trial of immediate versus deferred treatment with intravenous cidofovir, 48 individuals with AIDS, and with newly diagnosed retinitis, were given 5 mg/kg once weekly for 2 weeks as induction followed by 5 mg/kg once every other week. Cidofovir delayed the median time to CMV retinitis progression from 22 days to 120 days ( $P < 0.00001$ ).<sup>36</sup> Approximately 20% of patients had to stop the drug within

6 months or so because of dose-limiting nephrotoxicity. The most common adverse effects reported were proteinuria (23%) and neutropenia (15%).

A salvage study has been performed in which individuals with advanced AIDS who had failed therapy with standard ganciclovir or foscarnet were randomized to receive either 3 mg/kg or 5 mg/kg cidofovir as maintenance therapy. Again, a clear dose effect in time to progression was statistically significant.<sup>37</sup>

Although the drug must be given intravenously it has a prolonged intracellular half-life and persistent antiviral effect and so may be administered infrequently; suppression of urine cultures for 3 weeks or longer has been noted after a single dose of cidofovir. This also avoids the need for a permanent indwelling catheter. The current treatment strategy is 5 mg/kg once weekly for 2 weeks followed by a single dose of 5 mg/kg every 14 days (1 hour infusion).

Cidofovir is associated with serious nephrotoxicity which, unlike other antiviral agents for CMV disease, is only slowly reversible and may be permanent in some patients. The first sign of nephrotoxicity is usually proteinuria, at which point administration of the drug should be stopped immediately to avoid serious nephrotoxicity. Concomitant administration of probenecid and saline hydration has been shown to reduce the risk of nephrotoxicity with this drug, but it is essential to monitor serum creatinine and urine protein levels before each infusion.

## Investigational Agents

### 1263W94

1263W94 is a potent anti-CMV compound currently under clinical development. Although related to 2-bromo-5, 6-dichloro-1-(β-D-ribofuranosyl)-1 H-benzimidazole (BDCRB), 1263W94 does not inhibit the processing of CMV DNA. Instead, it directly inhibits the amount of viral DNA synthesis by a unique mechanism which is currently under investigation.

1263W94 has been well tolerated in Phase I studies in both healthy volunteers and HIV-infected volunteers. A Phase I/II multiple-dose escalation study in HIV-infected volunteers asymptotically shedding CMV is ongoing.

### Lobucavir

Lobucavir is a nucleoside analogue which has broad-range herpesvirus antiviral activity *in vitro* and is also active against HIV and hepatitis B. Phase I studies have shown the drug to have good oral bioavailability and research is currently underway to find a dose that achieves plasma levels which inhibit CMV.

### ISIS 2922

ISIS 2922 is an antisense oligonucleotide designed to inhibit the production of proteins required for CMV replication by targeting RNA molecules at multiple sites. *In vitro* studies have shown that when used in combination with other antiviral agents approved for the treatment of CMV disease, e.g. ganciclovir or foscarnet, ISIS 2922 has an additive antiviral effect.<sup>38</sup> Phase I/II trials have shown that this effect is translated *in vivo* when the drug is given intravitreally once a week for 4 weeks and

then once every 2 weeks. Phase III trials of ISIS 2922 in both early- and late-stage CMV retinitis patients are ongoing.

## GEM 132

This is an antisense molecule that is directed against UL36/37 of CMV that blocks the expression of these genes. It is currently in Phase II of clinical trials and is associated with dose-limiting toxicity.

## Monoclonal antibodies

Anti-CMV monoclonal antibodies directed against specific proteins may supplement the effect of antiviral therapy and prolong the time to relapse. Several antibodies are now in development (e.g. MSL-109 which appears to increase the time to relapse of CMV retinitis in individuals with AIDS).<sup>39</sup>

## Prophylaxis

### Ganciclovir

Two oral ganciclovir, placebo-controlled prophylaxis trials have been undertaken with contrasting results (Table 2).<sup>40,41</sup>

	Spector <i>et al</i> <sup>40</sup>	Brosgart <i>et al</i> <sup>41</sup>
Number of patients	725	994
Entry criteria	CD4 count < 50 cells/mm <sup>3</sup> or CD4 count < 100 cells/mm <sup>3</sup> and an AIDS defining opportunistic infection, positive CMV serology	CD4 count < 100 cells/mm <sup>3</sup> and positive CMV serology
Median CD4 count (cells/mm <sup>3</sup> )	22	34
Treatment	2:1 oral ganciclovir 1000 mg 3x/day versus placebo	2:1 oral ganciclovir 1000 mg 3x/day versus placebo
Median treatment time (months)	8.7	7.2
Median follow-up time (months)	9	9

Table 2: Comparison of oral ganciclovir prophylaxis trial designs and patient populations<sup>37,38</sup>

The results of the first trial showed a reduction in the risk of developing CMV end-organ disease. The onset of CMV retinitis was delayed by 8 months and there was a reduction in the risk of disease onset of 49% (20% for ganciclovir-treated patients compared with 39% for placebo) in the patients for whom analysis was possible (20%).<sup>39</sup> In the second trial there was a trend toward a reduced risk of CMV retinitis but it did not reach statistical significance.<sup>41</sup>

Differences between the two trials may account for the contrasting results. The Brosgart *et al* (1995) study did not perform ophthalmological examinations at baseline and patients only received follow-up eye examinations if they became symptomatic whereas Spector *et al* conducted detailed retinal examinations at baseline and every 2 months.<sup>40,41</sup> Median CD4 counts and length of treatment time also differed. Both studies had short follow-up periods so that the long-term outcome of the patients is not known.

## Aciclovir and valaciclovir

Several studies with oral aciclovir have been shown to result in enhanced survival in individuals with advanced HIV disease,<sup>42-44</sup> but failed to demonstrate the CMV prophylactic effect seen with intravenous aciclovir in bone marrow transplant recipients.<sup>45</sup> Valaciclovir is the L-valyl ester of aciclovir and when metabolized results in substantially higher plasma concentrations of aciclovir than are achievable with oral aciclovir. A trial was therefore designed to evaluate the effectiveness of valaciclovir in preventing CMV end-organ disease in individuals with AIDS and the impact of different levels of aciclovir exposure on survival.<sup>46</sup> The trial could not be performed in a placebo-controlled mode, first because many patients in Europe and Australia were already receiving aciclovir and second because there was concern that patients receiving placebo and investigators would be unblinded should they develop outbreaks of herpes simplex virus (HSV). A total of 1227 patients were enrolled with no CMV end-organ disease prior to study entry and CD4 counts less than 100 cells/mm<sup>3</sup> (median 31–33 cells/mm<sup>3</sup>). Patients were randomized to high-dose valaciclovir 2000 mg four times daily, high-dose aciclovir 800 mg four times daily or low-dose aciclovir 400 mg twice daily in the ratio 3:2:2.<sup>46</sup>

Time-to-event analysis of 184 confirmed CMV end-points (of which 79% were retinitis and 15% gastrointestinal disease [GI]) showed a significant protective effect for valaciclovir ( $P=0.03$ ). The incidence of confirmed CMV disease was reduced by 33% from 17.5% with aciclovir (high- and low-dose combined) to 11.7% on valaciclovir ( $P=0.01$  [Figure 3]). The reduction in CMV disease was most marked in patients with CD4 counts  $\geq 50$  cells/mm<sup>3</sup> ( $P=0.001$ ).

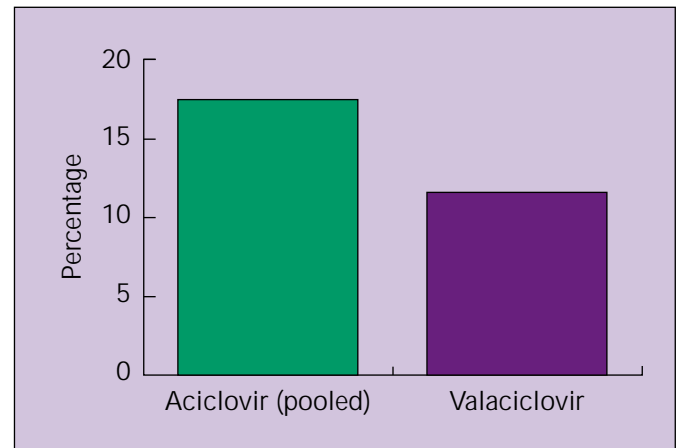


Figure 3: Incidence of confirmed CMV disease

Blood and urine samples from a pre-defined subset of patients enrolled in the trial were analysed by polymerase chain reaction (PCR) for CMV DNA and showed that valaciclovir has an impact on both CMV disease and virus shedding in blood and urine.<sup>46</sup> Only 51% of patients were negative for the virus in blood and urine at study entry and so were entering a true prophylaxis study. The greatest benefit of valaciclovir therapy in preventing the development of CMV disease was in patients who were PCR-positive in blood at study entry (Figure 4).<sup>47</sup> Time to CMV disease was also significantly longer in patients who had positive blood PCR results at study entry ( $P=0.01$ ).<sup>47</sup>

A non-significant trend toward decreased survival in the valaciclovir arm compared with aciclovir was noted.<sup>46</sup> The majority of deaths were attributed to HIV progression, the most common including lymphoma, bacterial pneumonia, disseminated *M. avium* complex, wasting syndrome and Kaposi's sarcoma. Deaths due to renal impairment and/or a thrombotic microangiopathy (TMA) syndrome were the only causes which occurred more frequently on valaciclovir than on the two aciclovir arms. There was also a higher rate of study drug discontinuation in the valaciclovir arm. When the primary reasons for this were analysed it appeared that patients in the valaciclovir arm were experiencing more GI intolerance.<sup>46</sup>

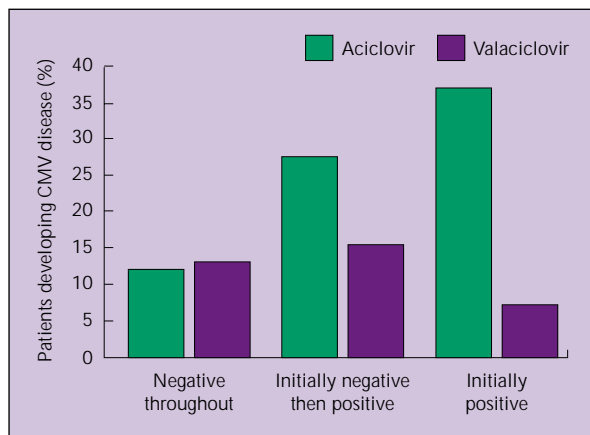


Figure 4: Relationship between CMV detection blood (PCR) results and CMV disease<sup>46</sup>

The dose of valaciclovir (8000 mg/day) may be at the limit of what patients could tolerate for prolonged periods. This is suggested by a higher incidence in the valaciclovir arm of a TMA syndrome which has some features of haemolytic uraemic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP). However, important features of the TMA-like syndrome reported in the study were different from *classical* TMA in several ways. For example, all patients had a gradual onset/less severe thrombocytopenia, less frequent neurological abnormalities and many had a concurrent medical condition that could account for the abnormalities attributed to TMA.

TMA-like syndrome occurred in patients with very advanced HIV disease (median CD4 count=14/mm<sup>3</sup>) who had been on study drug for a median of 54 weeks. In this time, patients on valaciclovir had been exposed to a total dose of almost 3 kg of drug, almost all of which would have had to be eliminated via the renal route. Patients developed renal impairment and haemolytic anaemia. Analyses are ongoing to determine whether the TMA syndrome was related to severe nephrotoxicity, a known side-effect of high-dose intravenous aciclovir. No cases have been found in studies of over 1000 HIV-infected individuals receiving valaciclovir or aciclovir for HSV suppression (up to 1000 mg of valaciclovir per day for 1 year) or in otherwise healthy individuals receiving suppressive valaciclovir (up to 1000 mg/day for 1 year) for genital HSV recurrences.<sup>48</sup>

The risk of HIV-associated TMA was increased in patients with a low CD4 cell count, with CMV disease and in those who have used fluconazole.<sup>49</sup> Several other drugs commonly used in patients with advanced HIV disease were also associated with a risk of a TMA syndrome in the ACTG204 study.<sup>50</sup> Whether this is associated with a direct effect of any of these drugs, or is a reflection of the fact that patients receiving these drugs have advanced HIV disease which itself predisposes to TMA-like syndrome is unknown. Patients with advanced HIV disease become readily dehydrated as a result of diarrhoea and fever and are very vulnerable to a modest decrease in renal function. These factors need to be carefully assessed in further studies to identify those patients who may be at risk of developing the TMA-like syndrome.

### Role of prophylactic therapy

An effective and safe prophylactic agent to prevent CMV disease is urgently required given the morbidity associated with disease and the serious side-effects of current therapies. However, there is still concern that indiscriminate long-term therapy in severely immunocompromised individuals may increase the risk of developing resistance.

With the newer quantitative methods of detecting CMV DNA or antigen in blood, it is hoped that a subset of patients may be defined who are at very high risk for developing CMV disease. These patients may prove amenable to prophylactic or pre-emptive therapy with the new oral antiviral agents.

## Summary and Management Recommendations

### Induction therapy

Clinical studies have shown that to halt the spread of retinal damage in CMV retinitis, induction therapy for a period of 2–3 weeks with either intravenous ganciclovir or foscarnet is required (*Category 1 Recommendation*) or it can be continued until ophthalmological improvement (*Category 3 Recommendation*).

Intravenous ganciclovir should be administered at 5 mg/kg every 12 hours (*Category 1 Recommendation*). Foscarnet can be given at 90 mg/kg every 12 hours (*Category 1 Recommendation*) or 100 mg/kg every 12 hours (*Category 2 Recommendation*).

Once control of the disease is confirmed by an ophthalmologist, life-long maintenance therapy is instituted to delay relapse.

### Chronic maintenance therapy

#### Intravenous ganciclovir or foscarnet

Standard maintenance therapy with either intravenous ganciclovir or foscarnet is used in patients to limit further retinal damage. Ganciclovir can be administered at a dose of 5 mg/kg once daily (*Category 1 Recommendation*) or 6 mg/kg 5 days/week or 10 mg/kg 3–7 days/week (*Category 2 Recommendation*). Foscarnet can be given at a dose of 90 or 120 mg/kg once daily (*Category 1 Recommendation*).

Patients with a relapse of Zone 1 retinitis after standard ganciclovir or foscarnet monotherapy or who show progressive CMV retinitis while undergoing monotherapy may be offered combination therapy (*Category 2 Recommendation*).

#### Oral ganciclovir

Oral ganciclovir maintenance therapy (500 mg every 4 hours or 1000 mg every 8 hours) is appropriate for use in patients without immediately sight-threatening (peripheral) lesions in whom the risk of more rapid disease progression is balanced by the benefit associated with avoiding daily intravenous infusions (*Category 1 Recommendation*).

Oral ganciclovir maintenance therapy should be instituted only after 2 weeks induction therapy followed by 1 week of maintenance therapy with intravenous ganciclovir and confirmation by an ophthalmologist that the disease is stable (*Category 1 Recommendation*). Caution should be advised about the use of oral ganciclovir in patients with Zone 1 disease as the oral formulation is probably not as effective as the intravenous and may allow more rapid disease progression (*Category 3 Recommendation*). Oral ganciclovir is less effective in patients who have received more than 100 days of prior intravenous ganciclovir treatment (*Category 2 Recommendation*) or who have had any prior retinitis progression (*Category 3 Recommendation*).

Oral ganciclovir has a bioavailability of 5% under fasting conditions and between 6–9% with food. It is therefore important that patients take their medication with meals.

#### Ganciclovir intraocular implant

The ganciclovir intraocular implant has the best efficacy to date of any CMV retinitis treatment and is appropriate therapy for treating newly diagnosed CMV retinitis in the

absence of extraocular CMV end-organ disease (*Category 1 Recommendation*). It is recommended for local control of unilateral Zone 1 disease in eyes with useful functional vision. (*Category 3 Recommendation*). The implant offers only local control of CMV disease and therefore it is recommended that some form of systemic therapy, should be used in addition to the implant to protect the untreated, fellow eye and other anatomical sites (*Category 3 Recommendation*), the risks versus benefits (Table 3) must be discussed with the patient (*Category 3 Recommendation*) as the device has been associated with some serious ocular adverse effects as a result of the surgical procedure.

## Benefits

- ◆ Very effective at controlling disease progression in the implanted eye
- ◆ Side-effects associated with intravenous ganciclovir are absent or reduced
- ◆ Suitable for patients non-compliant to intravenous ganciclovir

## Risks

- ◆ Temporary visual impairment (lasting an average of 20 days), due to astigmatism, occurs after the operation as a result of the procedure
- ◆ Protection does not extend to the contralateral eye or the rest of the body
- ◆ Implant must be replaced after 6 months
- ◆ Associated with retinal detachment or other vision-threatening ocular complications in about 10% of patients

Table 3: Ganciclovir intraocular implants – benefits versus risks

## Intravitreal therapy

Intravitreal therapy is recommended for local control of disease in patients unresponsive to, intolerant of or non-compliant with standard therapy who do not wish to undergo the surgical procedure associated with the intraocular implant (Table 4). Repeated intravitreal injections are associated with rare adverse effects including infection, haemorrhage and changes in intraocular pressure. As for the intraocular implant, protection does not extend to the other eye or the rest of the body and so some form of systemic therapy should also be considered (*Category 3 Recommendation*).

## Cidofovir

Intravenous cidofovir (5 mg/kg once weekly as induction for 2 weeks followed by a single dose or 5 mg/kg every 14 days) is recommended for treatment of first episodes or relapses of CMV retinitis in patients with normal renal function and no requirement

Drug	Dose	Recommendation
Ganciclovir	200–400 µg, 2–3 times/week for induction, then once weekly for maintenance	Category 2
Foscarnet	2400 µg in 0.1 ml, 2–3 times/week for induction, then once weekly for maintenance	Category 2

Table 4: Intravitreal drug regimens and Recommendation Categories

for the concomitant use of nephrotoxic medications (*Category 1 Recommendation*) and for patients for whom daily intravenous therapy is not feasible (*Category 3 Recommendation*). If there has been prior administration of nephrotoxic medications, an adequate wash-out period should be used.

Cidofovir is nephrotoxic and it is essential to monitor proteinuria and serum creatinine levels before each infusion. To reduce the risk of nephrotoxicity, cidofovir should be administered with probenecid and saline hydration (*Category 1 Recommendation*).

### **Individualized therapy**

There is currently no ideal therapy for CMV; all currently available systemic therapies are associated with toxicity and less than ideal efficacy. The treatment chosen should be individualized for each patient (*Category 3 Recommendation*); the choice is dependent on an informed discussion between the patient, clinician and ophthalmologist aimed at achieving a balance between efficacy, toxicity and quality of life.

## Resistance of Herpesviruses to Antiviral Drugs

All herpesvirus populations are composed of isolates with differing antiviral sensitivities. Therefore, if immunocompromised, increased viral burden ensues which is not totally amenable to inhibition of viral replication exhibited by antiviral drugs; there is a strong selective pressure for the emergence of isolates with altered sensitivities. These altered strains are termed resistant.

### Detection of Resistant Strains

The evaluation of potentially resistant herpesvirus isolates can be problematic because of the degree of genetic heterogeneity among these mutants. Furthermore, *in vitro* methods for the determination of the susceptibility of herpesviruses to antiviral drugs have not been standardized and the results can vary from study to study depending upon factors such as viral inoculum, cell line, culture media etc.<sup>1</sup> Because the IC<sub>50</sub> values with these different methods vary, it is important to be aware of which method is being used when susceptibility data are reported (Table 1).

Assay	IC <sub>50</sub> (μM)	
	Sensitive isolate	Resistant isolate
Dye-uptake	≤ 3	3–50
Plaque reduction	≤ 13	13–200

Table 1: HSV sensitivity to aciclovir as assessed by the dye-uptake and plaque reduction assays

Two *in vitro* assays are currently used to assess the susceptibility of herpes simplex virus (HSV) to antiviral drugs: the dye-uptake assay, primarily used in the USA, and the plaque reduction assay, primarily used in the UK. Both assays measure inhibition of viral cytopathic effect in the presence of antiviral drug. The dye-uptake assay is a semi-automated, colourimetric

method which can determine the *in vitro* susceptibilities of large numbers of clinical isolates based on the amount of red dye retained by viable cells. The plaque reduction assay is more labour intensive requiring the counting of plaques in a tissue culture dish, but is generally more accurate in predicting failure of antiviral therapy than the dye-uptake assay.<sup>2</sup> The assays have a relatively slow turn-around time of approximately 2 weeks.<sup>2</sup>

Both assays detect a proportion of isolates which appear to be aciclovir-resistant from people who have not been exposed to aciclovir. The dye-uptake method suggests that about 3% of viruses are resistant, whereas the plaque reduction assay suggests that the

Assay	Isolates (n)	Resistant variants (n)	Isolates resistant (%)
Dye-uptake (cut-off 3 μM)	1878	58	3.1
Plaque reduction (cut-off 13.0 μM)	1139	3	0.3

Data on file, Glaxo Wellcome

Table 2: Prevalence of aciclovir-resistant HSV in the immunocompetent population

figure is closer to 0.3% (Table 2). These figures show that the use of arbitrary definitions of resistance can give vastly different results which are difficult to relate to the clinical situation. However, work by Safrin *et al* (1994) suggests that the plaque reduction assay reflects the true clinical picture.<sup>3</sup>

The dye-uptake and plaque reduction assays may also be used for the detection of varicella zoster virus (VZV) isolates resistant to antiviral agents, but the Hybriwix assay is far more rapid (Table 3). This is a DNA–DNA hybridization assay that can be completed within 4–6 days. This assay can also be used to measure cytomegalovirus (CMV) susceptibilities to antiviral agents.

Ganciclovir is generally the first line therapy for CMV infections in the immunocompromised host and a number of isolates have been reported with varying sensitivities to the drug. In general, a strain with an IC<sub>50</sub> of ≤5 μM is considered sensitive and one with an IC<sub>50</sub> of >12 μM, resistant (Table 4).

IC <sub>50</sub> (μM)	
● Sensitive isolate ≤9	● Resistant isolate >40

Table 3: VZV sensitivity to aciclovir as assessed by the Hybriwix assay

IC <sub>50</sub> (μM)		
● Sensitive ≤5	● Partial resistance 6–12	● Resistant >12

Table 4: CMV sensitivity to ganciclovir as assessed by the Hybriwix assay

## Mechanisms of Resistance

Three mechanisms of HSV resistance to aciclovir have been described: thymidine kinase deficient mutants, thymidine kinase altered mutants and DNA polymerase mutants.<sup>4</sup> Because ganciclovir and penciclovir are analogues of aciclovir, the same mechanisms of resistance apply to these drugs as well. VZV resistance follows the same principles as HSV. CMV does not have a thymidine kinase but initial phosphorylation of ganciclovir to the monophosphate form is undertaken by a UL97 phosphotransferase. UL97 and DNA polymerase mutants have been identified which have altered the sensitivity to ganciclovir in CMV-infected cells. Foscarnet and cidofovir (HPMPC) differ from the nucleoside analogues in that they do not require phosphorylation by thymidine kinase for activation. However, they are substrates for the viral DNA polymerase so genetic changes in this protein can confer resistance.

The most common mutants in HSV- and VZV-infected cells are thymidine kinase deficient and account for approximately 95% of HSV isolates resistant to aciclovir.<sup>1</sup> This results in virus with either absent or markedly diminished phosphorylating activity and the inability to metabolize aciclovir to its active form. Fortunately, thymidine kinase deficient mutants have impaired replicative ability and a decreased ability to establish and reactivate from latency.<sup>5</sup> They are cross-resistant to drugs of similar structure such as ganciclovir and penciclovir, which also require thymidine kinase for their initial phosphorylation, but are sensitive to foscarnet and cidofovir (Table 5).

Far less common are resistant viral strains with an altered thymidine kinase, in which thymidine is phosphorylated but aciclovir will not serve as an enzyme substrate.<sup>6</sup> These isolates are susceptible to foscarnet but cross-resistant to ganciclovir.

Drug/mutation site	Sensitivity maintained	Cross-resistant
Aciclovir/thymidine kinase deficient	Foscarnet	Ganciclovir
	Cidofovir	Penciclovir
		Sorivudine

Table 5: Cross-resistance profiles of HSV and VZV thymidine kinase deficient mutants for antiviral drugs (based on *in vitro* data)

The third mechanism of resistance, which is only rarely documented, involves mutation of the viral DNA polymerase by which the enzyme is rendered resistant to aciclovir triphosphate and therefore allows viral replication to continue. This type of mutant is slower to evolve than the thymidine kinase deficient strains because this enzyme must still accept its natural substrate in preference to aciclovir triphosphate. DNA polymerase mutants are cross-resistant to foscarnet but retain sensitivity to ganciclovir.<sup>6</sup> Foscarnet DNA polymerase mutants have been described but maintain sensitivity to cidofovir and ganciclovir.<sup>7</sup> Cidofovir-resistant mutants have been generated *in vitro*.

## Epidemiology of Herpesvirus Resistance to Antiviral Drugs

Aciclovir has been the drug of choice for severe HSV and VZV infections for the past 15 years. In the immunocompetent host, the prevalence of aciclovir resistant strains is estimated at approximately 3%,<sup>8-10</sup> but there have been only a few reports of herpesvirus infections clinically resistant to aciclovir since the introduction of the drug in 1982.<sup>11-14</sup>

Fife *et al* (1994) reported on the post-therapy *in vitro* susceptibility of HSV isolates from a subgroup of immunocompetent patients who had received 6 years of suppressive aciclovir therapy for genital HSV disease.<sup>15</sup> Out of 113 isolates tested only four (3.5%) showed *in vitro* resistance, which was essentially identical to a 3.7% prevalence of aciclovir resistance in isolates tested pre-therapy.<sup>15</sup> These studies indicate that the prevalence of HSV resistance to aciclovir in the immunocompetent individual appears to have remained unchanged since the introduction of aciclovir, and even following prolonged administration of the drug there does not appear to be an increased incidence of resistance.

The situation in the immunocompromised individual is different, however, where although still uncommon,<sup>16</sup> the frequency of resistance to aciclovir and related drugs is increasing.

About 5% of treated, severely immunocompromised patients shed resistant virus;<sup>16-18</sup> the vast majority have been reported in bone marrow transplant recipients and individuals with advanced HIV disease. These isolates are normally thymidine kinase deficient mutants (approximately 95%). Hill *et al* (1991) reported on 100 HSV isolates taken from 51 HIV-infected individuals with herpetic lesions that did not respond to aciclovir.<sup>19</sup> Of these, 77 were aciclovir resistant by the dye-uptake method; 74 were thymidine kinase deficient and three showed a thymidine kinase altered phenotype. Similar rates of thymidine kinase deficient mutants have been detected in individuals

with different causes of immunodeficiency.<sup>9,10,19</sup> DNA polymerase mutants have only been described in isolated reports.<sup>20,21</sup>

The potential for the development of clinical resistance of HSV to aciclovir was realized shortly after the introduction of the drug.<sup>22</sup> Resistant VZV has evolved more slowly and has only been described in individuals with advanced HIV infection (CD4 counts generally <25/mm<sup>3</sup>) and following prolonged aciclovir exposure. Two studies evaluating aciclovir and sorivudine in parallel for the treatment of VZV infections have been conducted in HIV-infected individuals with an average CD4 count of 75/mm<sup>3</sup>. Out of a total of 105 isolates studied by *in situ* hybridization there were no cases of sorivudine resistance and only one case of aciclovir resistance.<sup>23</sup> Two studies have looked at VZV resistance to aciclovir in the HIV-infected individual and shown a similar pattern of resistance to that encountered with HSV (Table 6).<sup>2,19</sup>

	Kimberlin <i>et al</i> <sup>2</sup>	Hill <i>et al</i> <sup>19</sup>
Number of patients	33	51
Number of isolates	49	100
Isolates sensitive to aciclovir	25	23
Isolates resistant to aciclovir:	24	77
TK-	17	74
TK altered	5	3
DNA polymerase	2	–

Table 6: VZV resistance to aciclovir in HIV-infected individuals. TK, thymidine kinase<sup>2,19</sup>

To determine the prevalence of resistant CMV isolates in patients receiving ganciclovir, Drew *et al* (1991) prospectively monitored 72 culture-positive individuals with AIDS for the development of drug resistance.<sup>24</sup> No resistant strains were found in 31 patients before therapy or in patients who received ganciclovir for less than 3 months. Of 13 culture-positive patients treated for ≥3 months, five excreted virus resistant to ganciclovir. Overall, the prevalence of resistant virus was 7.6% after 3 months of therapy. All of the ganciclovir-resistant strains of CMV were sensitive to foscarnet.

Multiple human CMV strains frequently coexist in individuals with AIDS and may even exhibit dual resistance to antiviral drugs.<sup>25</sup>

### Transmission of resistant virus

A major question is whether drug resistant isolates are transmissible. Animal studies have shown decreased virulence and ability to induce latency in most thymidine kinase deficient phenotypes. Whether this observation will also apply to humans is unknown. There are no reports of person-to-person transmission of a thymidine kinase deficient mutant in either the immunocompetent or immunocompromised host and there has been only one report of transmission of a DNA polymerase mutant from an HIV-infected to an immunocompetent man.

### When is resistance not resistance?

In many cases, progression of CMV disease is not related to high-level resistance but

may result from other factors, such as inadequate drug delivery to the site of disease coupled with diminished susceptibility of the virus to a specific drug. In studies with ganciclovir maintenance, patients with relapsed retinitis usually respond to reinduction doses of ganciclovir which suggests that drug resistance is not a factor in the disease progression. Angiograms of the eye during acute retinitis show that the integrity of the blood vessels has been completely lost. As treatment starts to take effect, the vessels regain their pre-disease integrity. Therefore, acute retinitis disrupts the retinal barrier and may allow the drug to enter the affected area very easily. As the disease responds to therapy, integrity of the barrier is re-established and the dose of drug reaching the target site may be reduced. This hypothesis is supported by data showing that if the drug is delivered intravitreally, time to disease progression is approximately twice as long as seen with systemic therapy.<sup>26</sup> In conclusion, inadequate drug delivery may be responsible for treatment failure in some cases.



Figure 1: Genital HSV in an individual with HIV infection

## Management of Herpesvirus Recurrences in Populations at High Risk of Developing Resistant Infections

Factors which appear to predispose patients to the development of drug-resistant infection include severe and prolonged immunosuppression, repeated courses of antiviral therapy for episodic illnesses or prolonged therapy and inadequate treatment of acute disease.

### HSV

HSV lesions in the immunocompromised tend to be large, deeply ulcerated and can become necrotic (Figure 1). The appearance of satellite lesions is common and there is usually prolonged virus shedding. Areas most frequently involved are those where replication of HSV-1 or HSV-2 is expected, i.e. perirectal area, orofacial area, genitals and fingers.

In the HIV-infected individual most recurrences are sensitive to aciclovir and can be successfully treated. Patients should be re-evaluated 3–5 days after onset of treatment and if the response is good, therapy should be continued until there is evidence of complete healing (Figure 2). In the event of a poor response, the standard therapeutic dose should first be increased and the patient again re-evaluated after several days for evidence of healing (Figure 2). If there are concerns about compliance or the possibility of gastrointestinal (GI) disease impairing absorption of drug, the patient should be offered a course of intravenous aciclovir 1–2 mg/kg/hour as a continuous infusion.<sup>27</sup> Patients whose lesions are still not responding should have samples sent for susceptibility testing and be given intravenous foscarnet 40 mg/kg three times/day (Figure 2).<sup>28</sup>

Immunocompromised patients with aciclovir-susceptible recurrent HSV disease usually respond to aciclovir therapy within 7–14 days.<sup>29</sup> HSV lesions that are

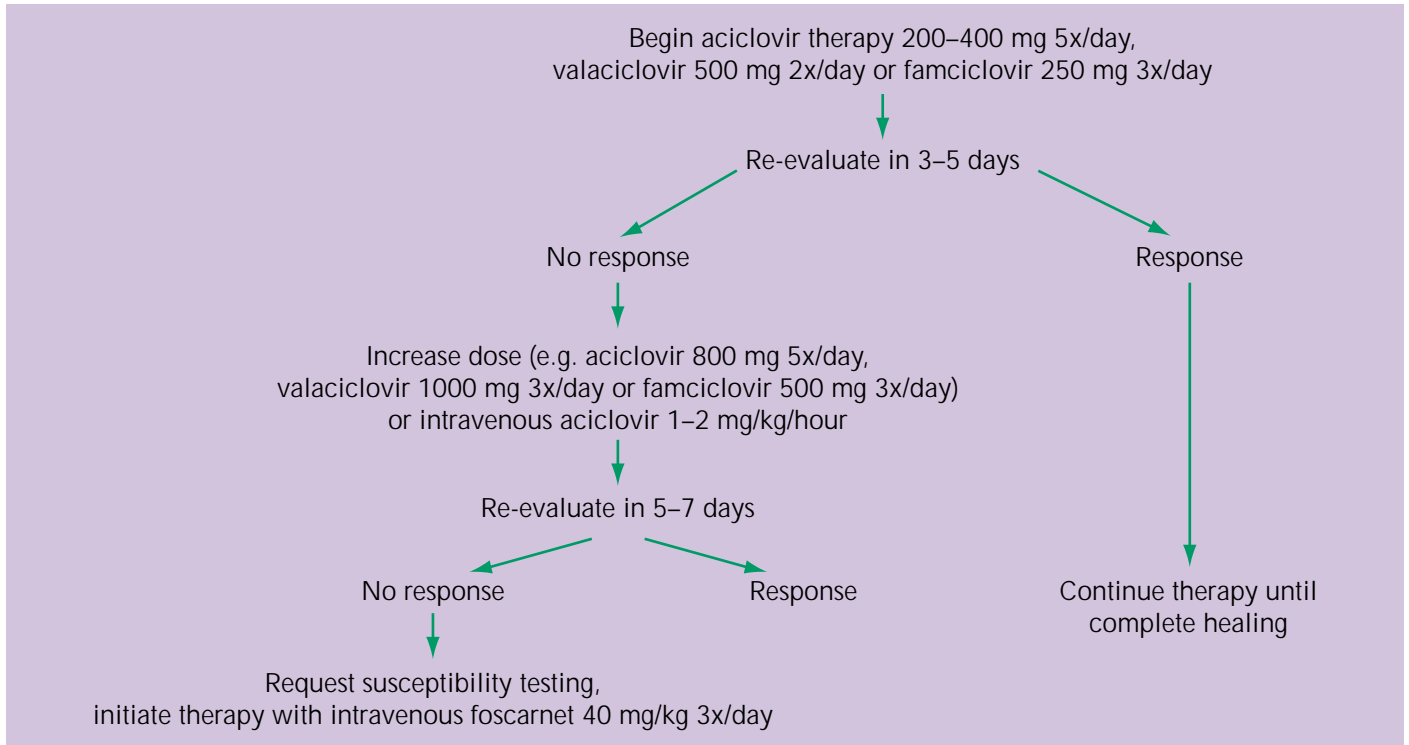


Figure 2: Management of HSV infections in immunocompromised individuals

unresponsive to 10–14 days of aciclovir therapy should be considered highly suspect in terms of aciclovir resistance. Antiviral susceptibility testing should be performed, if available, to document resistance and help guide further therapy.<sup>28</sup>

Once the acute episode has healed and the foscarnet therapy stopped, HSV may reactivate again. However, it is the wild-type which reactivates and so is fully sensitive to aciclovir.

### VZV

The clinical presentation of VZV infection in the HIV-infected individual may be either chronic varicella or herpes zoster. Patients suffer continued new lesion formation and a more severe dermatomal eruption than the immunocompetent host and healing is slow (Figure 3). In advanced HIV infection herpes zoster can be very severe and atypical. The lesions of verrucous herpes zoster occur in a limited area and are very long-lasting. Cutaneous dissemination may occur but visceral dissemination is not common even in individuals severely immunocompromised by HIV.

HIV-infected individuals with VZV resistant to aciclovir have CD4 counts  $<100/\text{mm}^3$  and generally  $<25/\text{mm}^3$ . Treatment options for resistant VZV infections are limited, although foscarnet is effective in accelerating healing. Approximately 22% of patients with AIDS who have had a previous episode of herpes zoster will suffer multiple recurrences.<sup>30</sup> Most of these episodes are responsive to a repeated course of standard therapy (such as aciclovir or valaciclovir [Figure 4]). In the event of progressive disease in a patient who is compliant but not responding to existing therapy, susceptibility testing should be undertaken and the patient given intravenous aciclovir followed by intravenous foscarnet if there is no evidence of healing (Figure 4).



Figure 3: Herpes zoster rash in an HIV-infected individual

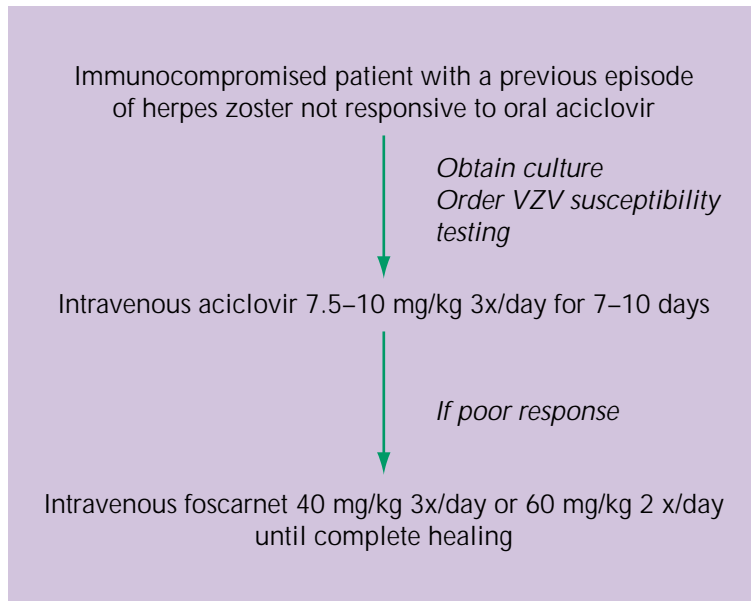


Figure 4: Management of VZV infections in immunocompromised individuals not responsive to oral aciclovir

Sorivudine may have a role in patients with cutaneously disseminated VZV or progressive retinal disease not responding to aciclovir or foscarnet therapy.<sup>31</sup> However, inadvertent co-administration of sorivudine with 5-fluorouracil in Japan resulted in several deaths and the USA Food and Drug Administration (FDA) have not approved sorivudine for use in the USA.

## CMV

The clinical presentation of CMV disease differs in individuals with AIDS compared with other immunocompromised patient populations. The most common manifestation is

CMV retinitis and the second is GI disease.

CMV resistance to therapy is difficult to detect clinically because progression of retinitis occurs with ganciclovir-resistant and -sensitive isolates and often in the absence of CMV-positive cultures. In addition, the eye provides a protected site and isolates may differ in their sensitivity pattern from samples taken from other areas of the body, e.g. blood or urine.

Quantitative PCR detection of CMV DNA can be used serially to evaluate treatment efficacy but is not widely available.<sup>32</sup> If levels of CMV DNA do not decrease or if they decrease but then increase again despite antiviral therapy, the emergence of resistance should be strongly considered. The presence of resistant virus should be assumed if blood or urine cultures are positive in a patient who is not responding to therapy.

If disease progression occurs while the patient is on standard maintenance therapy, e.g. ganciclovir, the patient should immediately receive induction levels of drug or combination therapy. If progression continues to occur alternative therapies must be instituted.

## Summary and Management Recommendations

As the number of immunocompromised patients increases, particularly those with advanced HIV disease, in whom the prevalence of HSV and the use of aciclovir is high, the incidence of aciclovir resistant disease should be expected to increase. Physicians involved with an individual case should seek expert advice. Every effort should be made to collect as much data as possible on these patients and enter them into controlled clinical trials. The impact of triple antiretroviral combination therapy has yet to be assessed.

At the present time, aciclovir resistance appears to be of little clinical significance for immunocompetent patients. Patients have been treated with prolonged courses of aciclovir without demonstrating an increased prevalence or incidence of aciclovir resistance.

Treatment of acute HSV or VZV in individuals with HIV infection should be continued until clinically active disease is no longer evident and complete healing has occurred. If HSV or VZV disease is unresponsive to therapy after 10–14 days, resistant virus should be suspected, and antiviral sensitivity testing performed. Foscarnet is effective in the treatment of aciclovir- or ganciclovir-resistant strains, but the emergence of foscarnet-resistant strains has also been documented. Cidofovir, a member of a new class of antiviral compounds, termed phosphonomethylethers, which have activity against a broad spectrum of DNA viruses, has been used successfully to treat disease in these patients.

CMV disease in individuals with AIDS requires aggressive management and follow-up because of the risk of progression even in the absence of resistant viral strains. If a patient fails to respond to induction levels of drug, alternative therapy, e.g. with cidofovir, should be instituted immediately. Combination therapy with ganciclovir and foscarnet may also be effective in patients with a relapse of retinitis or continued progression while undergoing sustained monotherapy (*Category 1 Recommendation*).

## CHAPTER 1

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## CHAPTER 1

**Figure 2** from Renne R *et al.* Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. *Nat-Med* 1996;**2**(3):342–346.

## CHAPTER 2

**Figure 1** from Sabin CA *et al.* The effect of CMV infection on progression of human immunodeficiency virus disease in a cohort of haemophilic men followed for up to 13 years from seroconversion. *Epidemiol Infect* 1995;**114**:361–372.

## CHAPTER 3

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## Recommendation Categories

### *Category 1 Recommendation*

Consistent evidence from controlled clinical trials. For example, for an antiviral this would be two properly randomized controlled clinical trials. In the case of laboratory tests, consistent evidence from comparative studies.

### *Category 2 Recommendation*

Evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytic studies (preferably from more than one centre), or from multiple time-series studies or dramatic results from uncontrolled experiments.

### *Category 3 Recommendation*

Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports of expert committees.

The *International Herpes Management Forum* (IHMF) World Wide Web site has information on the IHMF, forthcoming meetings and internet versions of *Management Strategies in Herpes*.

Internet address: <http://www.pps.co.uk/ihmf/welcome.htm>

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