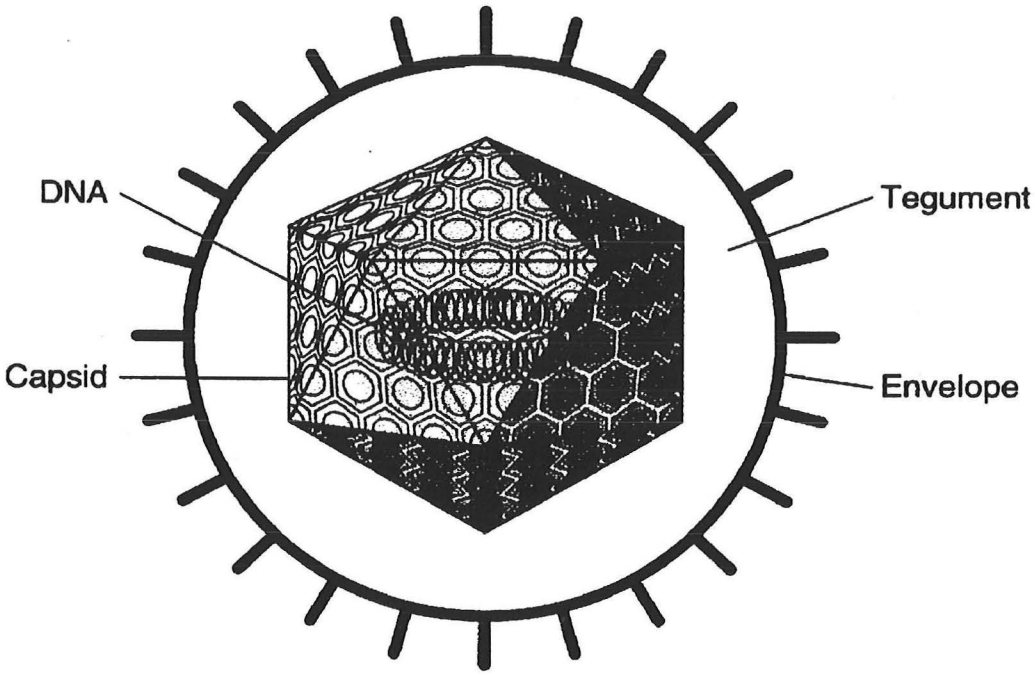
CMV Retinitis in the Era of HAART



Daniel J. Skiest, M.D.
University of Texas Southwestern Medical Center
Internal Medicine Grand Rounds
April 16, 1998

This is to acknowledge that Daniel Skiest has disclosed a financial interest or other relationship related directly or indirectly to this program with Gilead Sciences and Roche Pharmaceuticals.

Biographical Information

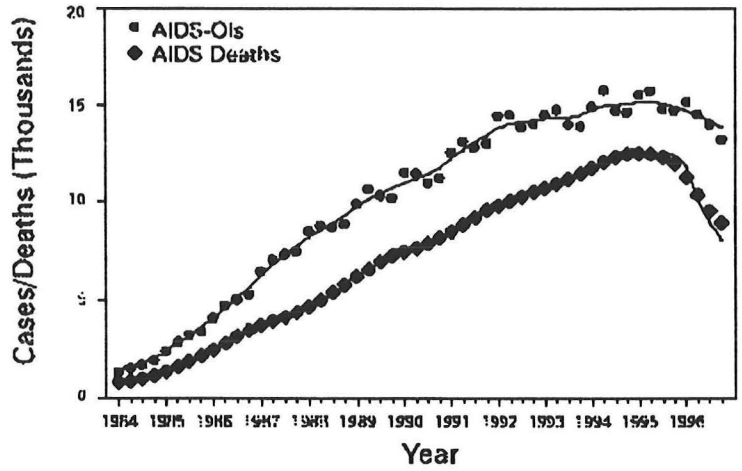
Name: Daniel J. Skiest, M.D.

Rank: Assistant Professor of Internal Medicine

Division: Infectious Diseases

Interests: Clinical aspects of HIV including CMV, diagnosis of CNS mass lesions, HIV in the elderly, and catheter infections

In the past few years dramatic advances have occurred in the management of patients infected with the human immunodeficiency virus (HIV). New concepts of pathogenesis and viral dynamics and a poweful new class of drugs, the HIV protease inhibitors, have led to improved outcomes for patients infected with HIV. In 1996, for the first time since the beginning of the AIDS pandemic, a reduction in AIDS-related mortality and opportunistic infections (OIs) occurred in the United States [1, 2].



Many patients receiving protease inhibitor based highly active antiretroviral therapy (HAART) undergo significant "immune reconstitution", with dramatic increases in numbers of CD4 lymphocytes. In addition to a decrease in the incidence of OIs, HAART has led to unusual manifestations of several OIs, including cytomegalovirus (CMV) retinitis.

The diagnosis, presentation, and management of CMV retinitis have undergone substantial changes in the past few years. These changes include the advent of new diagnostic modalities, alteration in the incidence and clinical presentation of CMV retinitis, and the approval of several novel therapeutic agents for treatment of CMV disease.

History

In the early 1900s Ribbert followed by Jesionek and Kiolemenoglou and others described the first cases of CMV inclusion disease, a devastating congenital infection characterized by low birth weight, hepatosplenomegaly, jaundice, thrombocytopenic purpura, and microcephaly. They concluded, incorrectly, that the typical intracellular inclusions were due to protozoans [3]. In 1921 Goodpasture and Talbot used the term "cytomegalia" to describe the typical cell enlargement and intranuclear inclusions in the liver and kidney of a 6 week old. It was soon realized that the pathologic changes were similar to the herpes viruses and that cytomegalic inclusion disease was most likely viral. This was confirmed in the 1950s when the virus was isolated independently in three different laboratories [4-6].

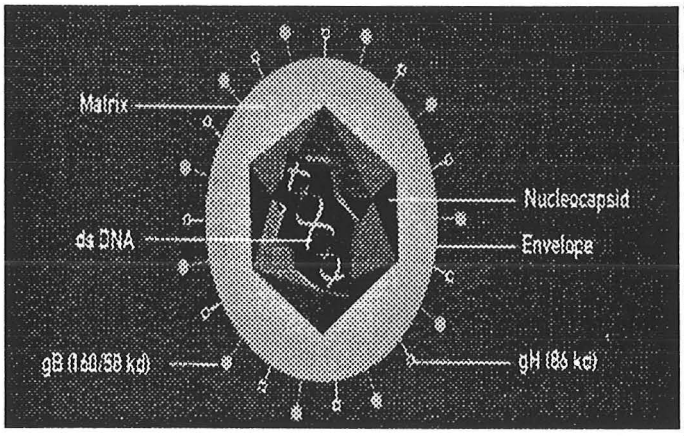
The first case of CMV retinitis in infants was reported in 1957 [7] and the first adult case of CMV retinitis was described by Foerster in 1959 [8]. Only twenty-one cases of CMV retinitis were reported in the literature before the 1980s, mostly in transplant recipients [9]. In the 1980s the incidence of CMV retinitis exploded with the advent of AIDS. After peaking in the early 1990s the incidence of CMV retinitis is now decreasing due to improvement in the management of HIV.

Virology

CMV belongs to the family of herpes viruses, which are DNA containing viruses, characterized by their ability to persist indefinitely in a latent form after acute infection, with subsequent reactivation during host immunosuppression. Herpesviruses are classified into three subfamilies based on the virus host range and other properties. The alpha-herpesviruses grow rapidly in many tissues. The betaherpesvirus, which include CMV, replicate in the host and in tissue culture relatively slowly and will only grow in specific cells types (human fibroblasts in the case of CMV). The gamma-

herpesviruses grow slowly in lymphoid cells. The newly described Kaposi's sarcoma associated virus (KSHV) is most closely related to the gamma subfamily [10].

Common Name	*Other Name*	*Subfamily*	*Genome size*	*Properties*
Herpes simplex virus 1	Human herpesvirus 1	α	152 kbp	short replication cycle, rapid growth in culture, latent in sensory ganglia
Herpes simplex virus 2	Human herpesvirus 2	α	152 kbp	
Varicella-zoster virus	Human herpesvirus 3	α	125 kbp	
Epstein-Barr	Human herpesvirus 4	γ	172 kbp	slow growth in lymphoid cells, immortalize lymphoid cells, limited host range
Cytomegalovirus	Human herpesvirus 5	β	229 kbp	long replication cycle, slow growth in culture, limited host range
Human herpesvirus 6		β	167 kbp	
Human herpesvirus 7		β	145 kbp	
Kaposis sarcoma associated herpesvirus	Human herpesvirus 8	γ	140 kbp?	difficult to grow in vitro



CMV is the largest of the herpesviruses, with a genome consisting of 229 kilobase pairs. CMV, like other herpesviruses, is composed of four structural elements: 1. the outer envelope which contains glycoprotein spikes and is derived from portions of the cell membrane, 2. the tegument (matrix) which consists of amorphous protein containing material, 3. the nucleocapsid, and 4. an internal core consisting of proteins and linear double stranded DNA.

Following initial infection, which is often asymptomatic, but can be associated with a mononucleosis like illness, CMV remains latent. Most individuals with prior CMV do not have any further sequelae, unless they become profoundly immunosuppressed. The exact site of latency of CMV is not definitively known, although circulating peripheral mononuclear and polymorphonuclear leukocytes are the most likely sites.

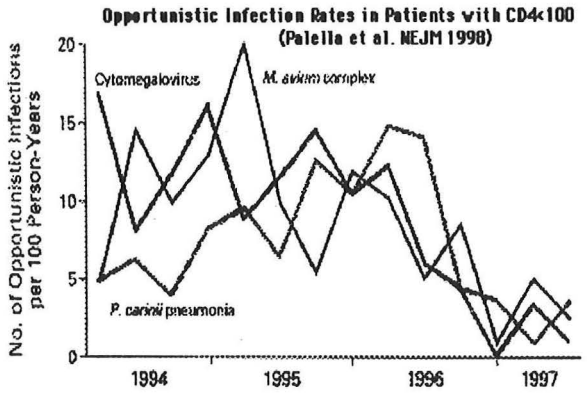
Epidemiology of CMV

Humans are the only known host for human CMV. Transmission occurs by direct or indirect person-person spread. The virus can be spread via contact with oral secretions, transnatally, during breast feeding, sexual contact, blood transfusion and organ transplantation [11, 12]. Primary infection can occur throughout life but most commonly occurs either early in life (in utero, at birth, or in the perinatal period) or during early adulthood presumably due to the onset of sexual activity. Transmission rates are high among young children in day care centers.

The prevalence of CMV antibodies in adults ranges from 40 to 100% and varies by location, sexual activity, sexual orientation and socioeconomic status [13-15]. Receptive anal intercourse is strongly correlated with CMV acquisition in gay men who may shed CMV in their semen or urine 7-40% of the time [16-18]. Homosexual men have the highest rates of seroprevalence at 90-100% [18] and often have persistent IgM antibodies, probably reflecting ongoing reactivation and

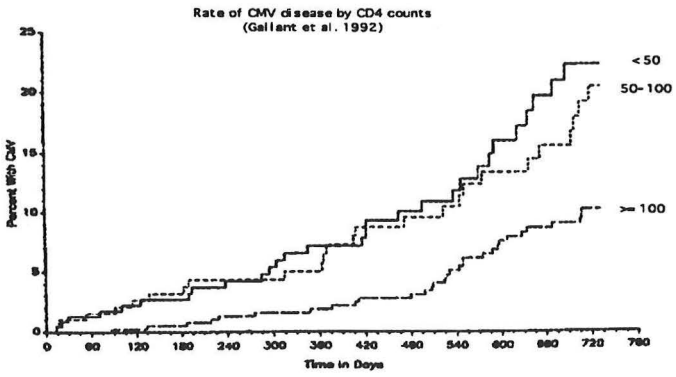
reinfection with new strains [11, 14]. HIV-infected patients with other HIV risk factors, generally have lower rates of seroprevalence [16, 18].

Incidence and Epidemiology of CMV Retinitis



Prior to the HAART era cytomegalovirus disease was diagnosed in 10- 45% of patients with late stage AIDS, while some autopsy series documented CMV in up to 90% of patients [19-22]. Several recent studies have demonstrated a declining incidence of CMV disease since the advent of HAART [2, 23-27]. At the Johns Hopkins HIV Clinic the relative risk of developing CMV disease in 1996-97 (when 63% received a protease inhibitor) compared to 1993-95 (when <1% received a protease inhibitor) was 0.21 [26]. Another possible explanation for the declining incidence of CMV retinitis could be the relative decrease in male-male sex as an HIV risk factor relative to other HIV risk factors.

Several clinical parameters have been associated with the development of CMV retinitis. The strongest predictor of developing CMV disease has been the absolute CD4 cell count. In studies performed in the pre-HAART era the risk of CMV disease directly correlated with lower CD4 cell counts; the median CD4 cell count for patients developing CMV retinitis ranged from 13 to 38 cells/μL [28-30]. In a study by Hoover et al. 81% of CMV retinitis diagnoses were made with a CD4 count < 50 [31]. In several large observational studies the risk of developing CMV disease at one and two years was 3.9-4.5% and 8.5%-15.5%, respectively, if the baseline CD4 count was less than 200-250 cells/μL [19, 29]. For patients with less than 100 CD4 cells/μL the risk of developing disease at 2 years increased to approximately 21% [19, 31].



Other consistent risk factors for the development of CMV retinitis include male-male sex as an HIV risk factor (probably because of the high seroprevalence of CMV), and a prior AIDS defining diagnosis [19, 22, 29, 32]. In a large study from Italy the risk of CMV was 15.3% at one year and 33% at two years, after an AIDS defining illness [22]. Other factors which have been associated with a risk for CMV disease in some studies include female sex, increasing age, receipt of *Pneumocystis carinii* prophylaxis, receipt of zidovudine, the presence of neurological disease and low CD8 counts [22, 29, 33].

More recently, HIV viral load, CMV viral load, and suboptimal antiretroviral therapy have been implicated as independent risk factors for CMV disease. The Multicenter AIDS Cohort Study followed 734 HIV-infected gay men, with under 500 CD4 cells and without an AIDS diagnosis, who had not received any antiretroviral therapy. In addition to a low baseline CD4 count, baseline HIV viral load correlated with the subsequent development of CMV disease. The relative hazard of

developing CMV disease at a viral load of 60-90,000 was 5.22 which increased to 8.80 for a viral load greater than 90,000 copies /ml [34].

Risk Factors for CMV Retinitis

CD4 cell count < 100 (esp. < 50)
High HIV viral load
Prior AIDS defining diagnosis
Male-male sex as HIV risk factor
Lack of protease inhibitor containing antiretroviral regimen
CMV viral load
CMV seropositive

Recent data indicate that optimal antiretroviral therapy can prevent several opportunistic infections, including CMV. In ACTG 320, a trial which randomized 1156 patients to either AZT + 3TC alone or AZT+ 3TC + indinavir, the CMV incidence rate per 100 patient years was 3.50 for the AZT + 3TC arm versus 1.31 for the protease inhibitor containing arm [35]. Recently, CMV viral load has also been shown to predict CMV retinitis in the short term (see below).

Interestingly, while retinitis is by far the most common manifestation of CMV disease in AIDS patients, accounting for approximately two-thirds of end organ disease, it is quite rare in other immunosuppressed populations, with high rates of CMV disease, such as recipients of allogeneic organ transplantation. African patients with AIDS also have low rates of CMV retinitis [36].

Immunity

Although the presence of CMV antibodies implies a history of past infection with CMV, immunity may not be complete. New infections with different strains can occur as well as reactivation of latent virus [14]. One study suggested that many patients with less than 100 CD4 cells/μL were deficient in antibodies to gpUL75 (gH) glycoproteins, which are thought to play a role in virus neutralization [37]. Others have suggested that patients whose T cells exhibit impaired specific proliferative responses to CMV antigens in vitro may be at highest risk for developing reactivation of CMV [38]. Certain HLA alleles including DR7, B44 and possibly B51 may also be associated with a predisposition to developing CMV retinitis in this population.

Histopathology

Infection with CMV results in specific histopathological findings. The cell infected depends on the the organ system involved, with epithelial and endothelial cells most commonly infected [11]. The characteristic microscopic changes include enlarged cells with intranuclear and intracytoplasmic inclusions, which contain viral particles. The intranuclear inclusion is often surrounded by a clear halo, giving the appearance of the pathognomonic "owl's eye" cell. Often there is a cellular infiltrate in proximity to the CMV containing cells, consisting of lymphocytes, plasma cells, and reticulum cells [14]. Confirmation that the changes are due to CMV can be accomplished by performing immunocytochemistry or in situ hybridization, as well as viral culture.

Pathologic diagnosis, usually with viral isolation is required for the diagnosis of CMV disease in most instances. However, due to the difficulty in obtaining retinal specimens and because the fundoscopic findings are characteristic, the diagnosis of CMV retinitis is primarily a clinical diagnosis. In the future laboratory markers may provide additional diagnostic information (see below).

Diseases Associated with CMV in HIV-infected individuals

Although CMV may infect virtually any organ in HIV-infected patients, retinitis is the most common clinical manifestation, accounting for 60-71% of cases [19, 22, 29, 31]. The next most common organ system to be involved is the gastrointestinal tract, including in order of involvement, esophagus, colon, hepatobiliary system, and pancreas. Neurologic involvement is being diagnosed more often as patients live longer and diagnostic tools improve. Neurologic

presentations include encephalitis, radiculomyelitis, and peripheral neuropathy. Other manifestations include adrenal disease, pneumonitis, and less commonly involvement of the spleen, genitourinary tract, and heart [22].

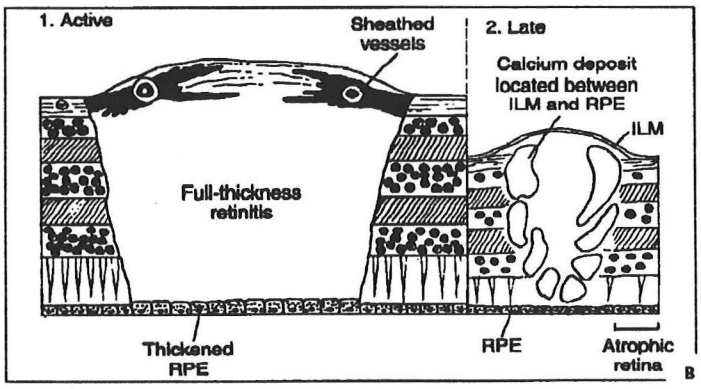
Anatomy of the Retina

The purpose of the retina is to convert and process photic energy into neuronal electrical signals that reach the visual cortex of the brain [39]. In order to accomplish this, the retina consists of nine layers. From the vitreous outward the retinal layers are: 1. the nerve fiber layer, 2. the ganglion cell layer, 3. the inner plexiform layer, 4. the inner nuclear layer, 5. the outer plexiform layer, 6. the outer nuclear layer, the external limiting membrane, 7. the rod and cone inner segment, 8. the rod and cone outer segment and 9. the retinal pigment epithelium. The retinal pigment epithelium is separated from the choroid layer by Bruch's membrane. The retinal pigment epithelial cells are joined by tight junctions, which restrict diffusion and form the outer component of the blood-retinal barrier. The inner component of the blood-retinal barrier is the endothelial lining of the inner retinal circulation [40].

Pathology of CMV retinal involvement

CMV retinitis results in full-thickness retinal necrosis with swollen retinal cells containing eosinophilic intranuclear and intracytoplasmic inclusions [41, 42]. Viral inclusions may be found in severals layers of the retina including the retinal pigment epithelium [11]. There is disruption of all layers of the sensory retina and pigment epithelium and associated hemorrrhage. A neutrophilic infiltrate may be present [43]. Usually there is an abrupt transition, between areas of necrotic and uninvolved retina. The lesions are restricted by Bruch's membrane and do not extend to the choroid [44]. Characteristically there is minimal vitreous inflammatory reaction [40].

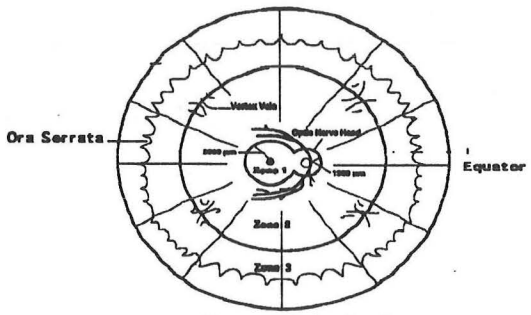
Infection of the retina occurs by hematogenous spread of the virus, following reactivation of latent virus. The virus disseminates primarily in mononuclear and polymorphonuclear leukocytes, and possibly endothelial cells. Early on infection is perivascular. The virus has been successfully grown in retinal pigment eptithelial cells in vitro [45]. Lesions enlarge and spread by cell-to-cell transmission of virus.



One possible explanation for the predilection of CMV for the eye in AIDS patients is the presence of cotton-wool spots. These areas of microinfarction in the nerve fiber layer are the most common fundus lesions in HIV, occurring in up to 50% of patients [46]. These lesions which resolve and recur regularly in AIDS patients are due to either deposition of circulating immune complexes in the microvasculature resulting in vessel occlusion, or active infection of vascular endothelial cells, causing ischemia and necrosis [47]. Cotton-wool spots are often present at the same location as later areas of CMV retinitis. It is thought that during periods of viremia, the virus may selectively crosses damaged blood vessels that are in proximity to cotton wool spots and retinitis forms in those areas [43]. This is supported by a recent study which found that CMV DNA could be detected in 90% of cotton-wools spots in patients without clinical evidence of CMV retinitis [48]. However, this explanation is not completely satisfactory, as there are patients with CMV retinitis who never had cotton-wool spots.

Diagnosis

The clinical presentation of CMV retinitis depends on the extent and region of retinal involvement. Common symptoms include floaters, flashes, blurred vision, decreased visual acuity, and scotomata. For the purposes of studies, the retina can be divided into three zones. Zone 1 is the area that is 3,000μm from the fovea and 1,500μm from the optic nerve. (The optic nerve head is 1500μm in diameter.) Zone 2 is a larger area, extending from zone 1 to the midequatorial region. Zone 3 extends from the border of zone 2 to the far periphery of the retina. Patients with a few lesions in the peripheral retina (e.g. zone 3) may be asymtpomatic. Lesions in zone 1 are considered sight threatening, since this zone contains the fovea and optic nerve head. Since there is usually little inflammation of the anterior chamber or vitreous, pain, redness and photophobia are unusual. At diagnosis ~30-40% will have bilateral disease [28, 49].



Zones of the Retina

Fundoscopic findings

Early lesions appear as white granular areas of opacifaction (due to retinal necrosis) in a perivascular distribution, representing the hematogenous route of infection. Later the lesions appear as fluffy, yellow-white exudates. There are areas of hemorrhage and edema, and sheathing of retinal veins may occur. Retinal edema is due to damage to tight junctions of the retinal microvasculature [50]. The appearance of the retina in active CMV retinitis has variously been described as "cottage cheese and ketchup", and "pizza pie". Roth spots (white centered flame shaped hemorrhages) have occassionally been described. The optic disc and fovea are usually spared, initially. Progression characteristically occurs at lesion borders. Later during the healing phase, scarring of the retina occurs, with pigmentation and atrophy. Mild anterior uveitis and vitritis (cells in the vitreous) may occur but are uncommon and if present are usually mild [12]. However, recently vitritis has been reported in patients treated with protease inhibitors (see below).

Serology is not useful in the diagnosis of CMV retinitis, since the majority of HIV-infected patients are CMV IgG positive. Blood and urine cultures are of limited utility in diagnosing CMV infection in AIDS patients; however, recent studies indicate newer laboratory tests may aid in the diagnosis. (see below)

Differential Diagnosis

A classic presentation of CMV is not difficult to diagnose, especially for an experienced ophthalmologist. However fundoscopic lesions are common in the HIV-infected patient, especially when profound immunosuppression is present. Cotton-wool spots can be differentiated by lack of associated hemorrhage and regression of the lesions rather than enlargement over a period of weeks. Two syndromes which are associated with varicella zoster virus and occassionally herpes simplex virus can occassionally be confused with CMV. Both syndromes present late in the course of HIV infection (CD4 < 100). Acute retinal necrosis presents with lesions in the peripheral retina and is associated with significant inflammation of the anterior chamber and vitreous. It starts unilaterally but often progresses to the fellow eye [51]. It may respond to antiviral medications in some patients. The other syndrome is rapidly progressive herpetic retinal necrosis, also referred to as progressive outer retinal necrosis (PORN). This syndrome causes a fulminant and usually bilateral necrotizing retinitis, with rapidly progressive multifocal lesions and minimal inflammation in the anterior chamber or vitreous [52]. In both syndromes retinal detachments and subsequent loss of vision are common. PORN is especially

devastating, since recovery of vision is rare even with systemic antiviral therapy. Other clinical entities in the differential diagnosis of CMV retinitis are listed in the table.

Differential Diagnosis of Retinal Lesions in HIV	*Features*
Cotton-wool spots	regress, do not enlarge
CMV	necrosis and hemorrhage, anterior chamber or vitreal inflammation unusual
Acute Retinal Necrosis (VZV or HSV)	lesions in peripheral retina, circumferential spread, prominent inflammation in anterior chamber and vitreous, fair-poor prognosis
Progressive Outer Retinal Necrosis (VZV or HSV))	bilateral, multifocal necrotic retinal lesions, starts in peripheral retina, anterior chamber or vitreal inflammation not present, very poor prognosis
Toxoplasmosis	indurated appearance with sharp demarcation of borders, hemorrhage rare, vitritis common
PCP	choroidal involvement with yellow-white subretinal plaques
Crytpoccocus	multifocal, discrete, yellow-white lesions of choroid or retina, often associated with meningitis
Candida	fluffy discrete lesions, vitritis but no hemorrhage (uncommon without other risk factors)
Syphilis	usually marked anterior chamber and vitreal reactions

ref. [51-53]

Clinical Course

Untreated CMV retinitis is associated with progression of lesions within 3-4 weeks, bilateral involvement and blindness in four months. Lesions expand outwardly from border areas or may involve new areas of the retina. The median rate of progression of lesions toward the fovea was measured at 24 microns per day in untreated patients [54]. As the lesions expand outwardly, the original areas of involvement become atrophic and have a "burned out" appearance, leading to loss of function and thus vision of this area of retina. Involvement of the contralateral eye during treatment has been associated with the recovery of resistant virus [55].

Complications

Following retinal necrosis the retina is left thin and atrophic and is susceptible to tears, resulting in retinal detachment. This complication can occur either during active retinitis or in healed retinitis. The risk of retinal detachment at six months is approximately 11%, while at one year the risk varies from 24% to as high as 50% [32, 56, 57]. Factors associated with an increased risk of detachment include peripheral retinal involvement, amount of retinal involvement (up to 50%), and retinitis activity on the most recent examination. The classic presentation of a detachment is the sensation of a curtain coming down over the eye. Other symptoms include the sudden onset of floaters, flashes, loss of visual field and decreased acuity. The retina can be surgically reattached by means of a vitrectomy with injection of silicone oil to tamponade the retina. The surgery is successful in approximately 90% of cases [58]. However, postoperative visual acuity is often inferior to pre-detachment acuity [54] and cataract formation is an important long-term complication [59].

Survival

Several studies have documented a decreased survival in patients diagnosed with CMV disease [19, 22]. In 1992 CMV disease accounted for 9.9% of deaths in patients with AIDS [20]. In large observational studies from the pre-HAART era, the median survival of patients with a CMV

diagnosis was 6 to 8 months [19, 22]. The survival of patients in the HAART era is not known, but it is probably substantially longer in comparison to the pre-HAART era.

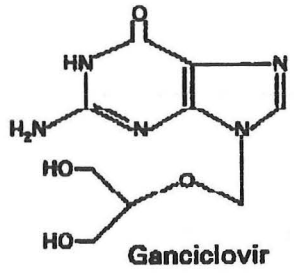
TREATMENT

Systemic Therapy

The primary goal of treatment is to preserve vision by arresting progression of retinal disease. With the currently available agents, which are virustatic, CMV viral replication is prevented but the virus is not eliminated. With successful treatment an active lesion is converted to an atrophic and gliotic scar. Recent advances in treatment include parenteral agents and local therapies, both of which have been shown to have improved efficacy over conventional therapies. Whereas two years ago only two drugs were available for treatment, there are now five drugs available and several on the horizon.

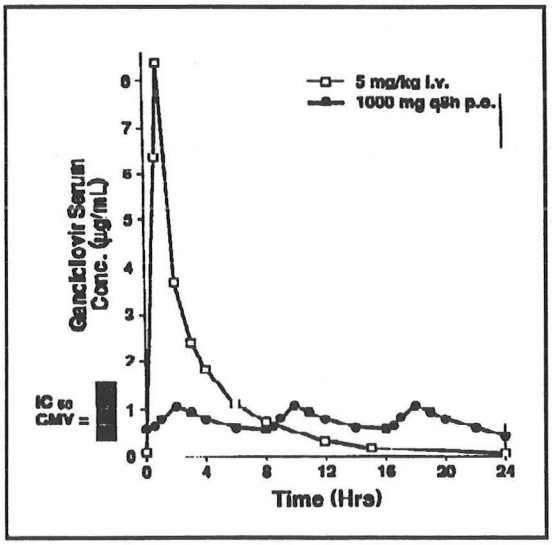
Ganciclovir

Ganciclovir (DHPG), a guanosine analogue with activity against all herpesvirus *in vitro*, was the first effective drug to be used for treatment of CMV [60]. It works by inhibiting viral DNA polymerase by competitively inhibiting the incorporation of deoxyguanosine triphosphate into elongating viral DNA, thus slowing DNA replication. It must be converted intracellularly to ganciclovir triphosphate. The first phosphorylation is by a viral protein kinase, a product of the UL97 gene, while the second and third phosphorylations are conducted by cellular kinases. The drug is virustatic against CMV.



Ganciclovir

Following the intravenous infusion of 5 mg/kg the peak serum ganciclovir concentration is 32 μM; twelve hours post infusion the serum concentration is less than 2.0μM [60]. The oral formulation of ganciclovir is poorly bioavailable, with only 3-9% absorption [60, 61]. After the usual oral dose of 1000 mg three times a day the peak serum concentrations is 4.8uM, with a trough of 0.8μM. The estimated median effective inhibitory dose (ED_{50}) is about 0.4-7.2 uM [60, 62, 63]. Thus with oral ganciclovir serum drug concentrations may be at or below the ED_{50}. A recent study indicated that the area under the time-concentration curve correlated with efficacy but the peak concentration did not [64].



The standard intravenous ganciclovir regimen for CMV retinitis consists of "induction" with 5mg/kg IV twice daily for 14-21 days, followed by maintenance therapy at 5mg/kg/day. Induction therapy with intravenous ganciclovir usually results in stabilization of fundus lesions within two weeks, with complete response in one month. Side effects of ganciclovir are mostly due to bone marrow suppression with neutropenia, thrombocytopenia, and anemia. The neutropenia which can occur in up to 25% of patients can be ameliorated with the use of colony stimulating factors. Other less common side effects include confusion, dizziness and azospermia.

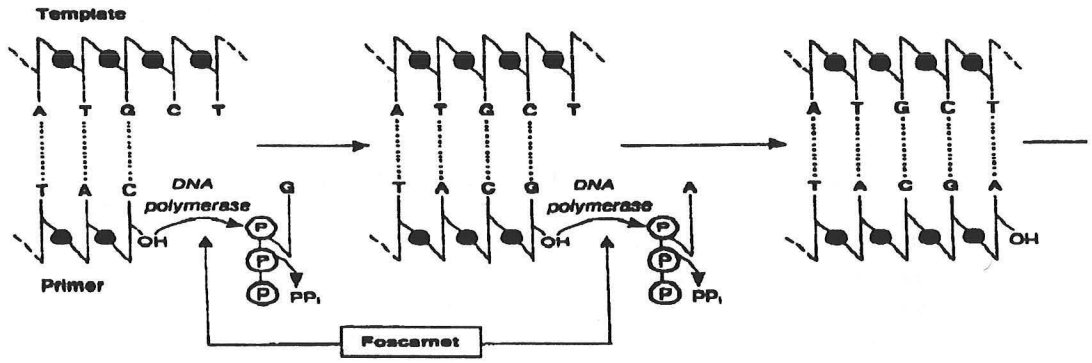
Oral ganciclovir, is approved for maintenance therapy, although its efficacy is limited somewhat by its poor

absorption. The approved dosage for maintenance is 1000 mg three times per day. In general times to progression are somewhat shorter in patients who receive oral ganciclovir compared to the IV formulation. In several studies comparing oral to intravenous ganciclovir the mean time to progression, based on masked assessment of fundus photographs (the standard endpoint used in clinical studies), for oral ganciclovir ranged from 51 to 58 days compared to 62 to 81 days for intravenous ganciclovir. Based on non-blinded fundsocopy mean times to progression for oral ganciclovir ranged from 68 to 86 days versus 96 to 109 days for IV ganciclovir [59, 64, 65].

A recent randomized prospective study investigated the efficacy of higher doses of oral ganciclovir compared to standard intravenous ganciclovir for maintenance of CMV retinitis. This study found that higher doses of oral ganciclovir (4.5 and 6.0 gm/day) were statistically equivalent to IV ganciclovir 5mg/kg/day, but were associated with fewer catheter related side effects such as sepsis [64].

The side effects of oral ganciclovir are generally similar to the IV formulation, although some patients may have gastrointestinal complaints, and a few studies have reported mild elevation of serum creatinine [64, 66]. The disadvantages of the oral form include the lower efficacy and the theoretical concern that low serum and intravitreal drug levels may encourage the emergence of resistance. These disadvantages must be weighed against the benefits, which include convenience and a lower incidence of catheter related complications including sepsis, since patients on oral therapy do not require an indwelling venous catheter. Most clinicians would consider oral ganciclovir for patients with peripheral, non-sight threatening retinitis which has been easily stabilized after induction therapy. Patients who have had a good response to HAART are also logical candidates for oral ganciclovir.

Foscarnet



Foscarnet-mechanism of action

Foscarnet (phosphonoformic acid) is a pyrophosphate analog which does not require phosphorylation. Thus most CMV resistant isolates remain susceptible to foscarnet. It works by binding directly to the pyrophosphate binding site of the DNA polymerase. It prevents cleavage of pyrophosphate from deoxyadenosine triphosphates. The ED_{50} for foscarnet has been variously defined as 100-190μM [63], 120μM [54], 300μM [67], and 400μM [68] . The mean peak and trough plasma concentrations of foscarnet following intravenous induction therapy are 88μM and 93μM, respectively.

Initial therapy consists of "induction" with 90mg/kg IV BID for 2-3 weeks followed by maintenance therapy of 90-120mg/kg/day. Using the higher maintenance dose of 120mg/kg/day may result in decreased rates of progression [69]. The side effects of foscarnet are mainly due to the fact that it is an anionic compound which binds divalent cations, such as calcium and

magnesium. Side effects include nephrotoxicity (reversible), hypocalcemia, hypokalemia, hypomagnesemia, hypophosphatemia, malaise, nausea, genital ulcers in males, and parasthesias. Hydration with saline is required to minimize renal toxicity. Seizures were intially reported with foscarnet but have not been reported in recent larger trials [70-72].

In the only large, head to head trial of ganciclovir versus foscarnet for treatment of newly diagnosed CMV retinitis the median time to progression by fundus photographs was 56 days for ganciclovir and 59 days for foscarnet [71]. Interestingly, a modest survival benefit of four months was noted for foscarnet. Reasons for this benefit are not clear but may include the fact that foscarnet has modest anti-HIV activity and fewer patients in the ganciclovir arm received zidovudine due to concomittant myelotoxicity. Although foscarnet was associated with a survival benefit in this study, it was also associated with more side effects; substantially fewer patients could tolerate foscarnet compared to ganciclovir [73]. Foscarnet was associated with a survival benefit in another small study; however, this study used historical controls [74]. Other studies comparing ganciclovir to foscarnet have not demonstrated significant differences in survival [72]. The possible modest survival benefit observed with foscarnet may not be relevant in the current era of HAART.

In the pre-HAART era, progression of CMV retinitis occurred in virtually all patients despite continued maintenance therapy. The postulated reasons for this include the poor host immune response, development of viral resistance, inadequate serum drug concentrations and indadequate drug delivery to the retina. Since CMV retinitis is a local manifestation of a systemic disease, the concentration of drug in the vitreous may be more important than systemic drug levels. Drugs used for treatment of CMV retinitis must penetrate the blood retinal barrier to provide drug concentrations capable of inhibiting viral replication. This does not appear to be a major problem in the treatment of active retinitis however, because of breakdown of the blood-retinal barrier. In the few instances in which it has been studied, vitreous drug concentrations for ganciclovir and foscarnet have paralleled or exceeded simultaneous serum levels [63]. In addition, the vitreous half-life of these drugs appears to exceeds plasma half-life, thus providing longer exposure times.

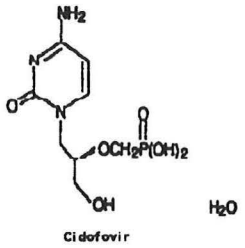
Ganciclovir Resistance

The definition of viral resistance to the various anti-CMV drugs is not well standardized, due to different definitions of in vitro resistance, the fact that patients may shed several CMV strains in their blood or urine and the difficulty of obtaining intravitreal viral samples. Nevertheless studies have elicited the major mechansisms and the incidence of clinical resistance. Most ganciclovir resistance is due to a mutation of the CMV UL97 gene, which encodes a protein kinase. Thus the drug can not be efficiently phosophorylated. The incidence of resistance correlates with time on treatment [55, 75, 76]. Isolates from patients treated with ganciclovir for less than 3 months are rarely resistant to ganciclovir, while 8-31% of those treated for longer than 3 months, may grow resistant virus [68, 75, 77]. Patients receiving prolonged therapy (> 9 months) can occassionally develop high level resistance, which is associated with mutations in the UL97 gene as well as the DNA polymerase gene [76]. Low level ganciclovir resistant (due to the UL97 mutation) isolates can usually be treated with cidofovir; however, strains with alterations in the DNA polymerase may be cross-resistant to cidofovir and occassionally to foscarnet. Recent studies have indicated that the incidence of in vitro resistance to foscarnet and cidofovir also increases with time on treatment [78].

Recently, two new agents have been approved for the treatment and maintenance of patients with CMV retinitis. Addition of these agents to our armamentarium has greatly improved our ability to treat patients.

NEW THERAPIES

Cidofovir



Cidofovir

Cidofovir is a nucleotide analogue of deoxycytidine monophosphate with activity against all human herpesviruses. It is converted by cellular kinases intracellularly to cidofovir diphosphate, which is the active metabolite [79]. It has a prolonged intracellular half-life (17-30 hours), which allows infrequent dosing and obviates the need for a permanent indwelling central venous catheter. Induction therapy is 5 mg/kg once a week for 2 weeks then 5mg/kg every other week for maintenance. In a study of 48 patients with previously untreated peripheral CMV retinitis randomly assigned to cidofovir versus deferred therapy the median time to progression (by masked fundus photography) was 120 days versus 22 days for deferred therapy group [80]. A second randomized controlled trial compared low dose cidofovir (3mg/kg) and standard dose cidofovir (5mg/kg) to deferred therapy for patients with untreated peripheral retinitis. The times to progression were significantly prolonged in the two cidofovir arms compared to the deferred therapy groups [81].

A third trial in which cidofovir was used for the treatment of patients with a retinitis progression or with intolerance to either ganciclovir or foscarnet, reported a median time to progression of 115 days for patients receiving 5 mg/kg of cidofovir. However, the median time to progression or drug discontinuation (i.e., due to adverse effects) was only 49 days [82]. This latter figure is probably more clinically relevant, since if patients can not tolerate the drug, they will not benefit from its prolonged efficacy.

A theoretical concern with intravenous cidofovir has been the apparent lack of effect on CMV viremia. In the two phase III trials of intravenous cidofovir, patients receiving the drug continued to have viremia throughout the study period [80, 81]. In contrast patients treated successfully with foscarnet or ganciclovir usually clear their viremia [70]. Whether the persistent viremia with cidofovir has any clinical significance is not known.

Cidofovir has a narrow therapeutic index and a significant side effect profile including proteinuria, significant renal insufficiency, iritis and hypotony (decrease in intraocular pressure) and neutropenia. Nephrotoxicity occurs due to injury to the proximal convoluted tubule cells. Cidofovir is concentrated via active transport in the proximal convoluted tubule cell by an anion transporter in the basolateral membrane. Since export of cidofovir at the luminal membrane is less rapid than uptake, high concentrations are attained in the proximal tubule cell [83]. Clinically the nephrotoxicity appears similar to Fanconi syndrome with proteinuria, glucosuria, bicarbonaturia, phosphaturia, polyuria and decreased glomerular filtration rate. Hydration and administration of probenecid, which competes with cidofovir for uptake in the proximal tubule cell, has been shown to decrease the incidence of nephrotoxicity [83, 84]. Probenicid, however, may not be tolerated in a substantial number of patients due to nausea, vomiting, headache, fever, flushing, angioedema and rash. In one study 56% of patients had adverse reactions to probenicid [80].

Although rarely reported in the two largest pre-approval studies of cidofovir, iritis and hypotony are important complications of parenteral as well as intravitreal treatment. In a recent case-control study by Davis et al, the incidence of iritis and hypotony was 26% and 9%, respectively [85]. Because of the substantial toxicity cidofovir should only be administered in a closely monitored setting, such as an infusion clinic, to assure patient compliance with hydration, receipt of probenecid, and adequate renal function prior to infusion.

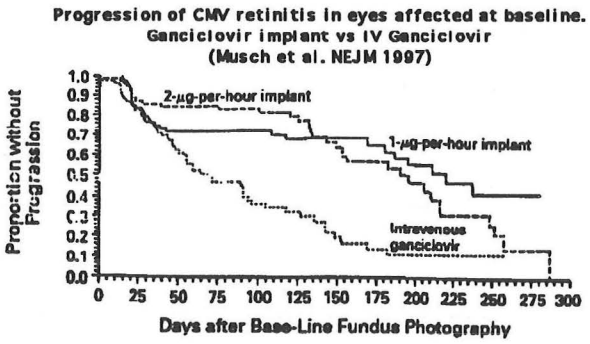
Local Therapy

Local therapy is attractive because it can achieve high levels of drug at the site where the infection occurs. The available forms of local therapy include direct intravitreal injections of drugs (ganciclovir, foscarnet) and the ganciclovir implant.

Intravitreal ganciclovir (200-400ug) or foscarnet (1200-2400ug) can be administered two to three times weekly for 14-21 days and then once weekly as maintenance therapy. While intravitreal injections are generally successful they are inconvenient and can be associated with endopthalmitis, retinal detachment and vitreous hemorrhage [86-89]. In most cases, due to the inconvenience and complications, intravitreal therapy is restricted to those patients who are intolerant of systemic therapy, can not receive the ganciclovir implant, or have failed systemic therapy.

Ganciclovir Implant

The ganciclovir implant (Vitrasert) represents a signficant advance in the management of CMV retinitis. The implant contains a slow-release pellet containing 6mg of ganciclovir compressed into a 2.5mm disk. This disc is surrounded by a drug-permeable polymer, polyvinyl alcohol (PVA), which is surrounded by drug-impermeable ethyl vinyl-acetate. The rate of drug delivery is controlled by varying the area of the PVA diffusion port. It is sutured into the posterior segment of the eye through a small incision in the pars plana a relatively avascular area. It is usually inserted under local anesthesia. Ganciclovir is released at a rate of 1 ug/hour, achieving a mean intravitreal concentration of 4 ug/ml (16uM) which is about 4 times higher than with IV ganciclovir [90] . The drug concentration remains high continuously, thus avoiding times when no drug is present in the vitreous, as occurs with systemic drug administration. By avoiding low troughs, resistance may be less of a problem. The reservoir contains enough drug to last approximately 7 to 8 months, after which time it can be replaced [91]. In two trials the median time to progression after insertion of the implant ranged from 221 to 226 days, which is substantially longer than other therapies [90, 92]. The risk of progression for intravenous ganciclovir compared to the implant was 2.3 [92].



One of the primary advantages of local therapy is that since systemic drug concentrations are neglible, systemic side effects do not occur. However, CMV is a systemic disease and patients with CMV retinitis are often viremic. Thus, patients treated with local therapy exclusively are at risk for fellow eye involvement as well as extraocular disease [90, 92]. A recent trial addressed these issues by randomly assigning patients with CMV retinitis to receive intravenous ganciclovir, implant with oral ganciclovir or implant with placebo. This study demonstrated a significant decrease in contralateral eye involvement and a strong trend towards decreased extraocular disease in patients receiving oral ganciclovir compared to oral placebo [93]. Patients treated with oral ganciclovir plus the implant also had longer times to recurrence in the affected eye. However, in a sub-group analysis of patients who received protease inhibitor therapy, no differences were seen among the treatment groups, presumably because the protease inhibitor was so effective in preventing recurrence. At present most clinicians would recommend continuing oral ganciclovir in patients with an implant; however, in patients with inactive retinitis, an excellent response to HAART and undetectable CMV DNA, some may consider discontinuing oral ganciclovir.

Complications with the implant have been infrequent and include occassional endophthalmitis and vitreous hemorrhage and a higher incidence of early retinal detachment [90, 92]. However, the cumulative rate of retinal detachment is probably similar in patients receiving the implant compared to those treated with other therapies. In addition patients often experience transient blurred vision for 2-4 weeks after the implant is inserted, due to surgery induced astigmatism. Since oral ganciclovir is required along with the implant, the usual toxicities of oral ganciclovir are also important to consider. Due to its superior efficacy with relatively few side effects, the implant is probably the treatment of choice in patients with clinically significant CMV retinitis

Intravitreal cidofovir has been studied for the treatment of newly diagnosed retinitis as well as for refractory retinitis. It can be administered on an infrequent dosing schedule (20μg every 5 to 6 weeks) because of its long half-life. It has good efficacy with the median reported time to progression after a single injection of 55 to 64 days [84, 94]. However, because of toxicity the drug has not been approved by the FDA. Iritis and hypotony have occurred in a significant number of patients studied [84, 94-97]

Drugs for Maintenance Treatment of CMV Retinitis

Agent	Dose	Median Time to Progression (photos)*	Main Toxicity	Comments
IV ganciclovir	5mg/kg QD	47-70d	neutropenia, thrombocytopenia catheter related complications	Longest clinical experience, catheter required
Oral ganciclovir	1g TID	29-50d†	neutropenia, thrombocytopenia	no catheter required, less efficacious, consider higher dose
IV foscarnet	90-120 mg/kg QD	40-59d	nephrotoxic, electrolytes (Ca, Mg), malaise, seizures ?, catheter related complications	catheter required, hydration required, reserve for salvage therapy
IV cidofovir	5mg/kg Q O wk	120d	nephrotoxic, iritis, hypotony, neutropenia	probenecid and hydration required, careful monitoring required no permanent catheter required, convenient dosing
Ganciclovir implant	1 implant (1 μg/hr)	226d	endopthalmitis (rare), early retinal detachments	oral ganciclovir required to prevent contralateral and extraocular disease
Intravitreal Ganciclovir	200-400μg/wk	50-103d	endopthalmitis, retinal detachments	inconvenient, does not protect contralateral eye nor prevent extraocular disease
Intravitreal Foscarnet	1200-2400μg/wk	?	endopthalmitis, retinal detachments	inconvenient, does not protect contralateral eye nor prevent extraocular disease

*Time to progression from start of induction therapy

†Time to progression from start of maintenance therapy

The choice of induction and maintenance therapy for patients with CMV retinitis needs to be decided on an individual basis. Several factors need to be weighed including efficacy, site of retinal involvment (zone 1 versus peripheral), convenience and quality of life, and toxicity. One of the most important aspects of therapy is optimization of the antiretroviral regimen. Control of HIV replication and restoration of immune function may be more important than the choice of specific CMV agent. In the past, all patients required maintenance therapy. However, now some clinicians may consider stopping maintenance therapy in certain patients with excellent responses to HAART and quiescent retinitis (see below).

Treatment of Recurrent Retinitis

Recurrence of retinitis may be due to inadequate drug levels, resistance, or poor host response. Studies have demonstrated *in vitro* synergy with the combination of foscarnet and ganciclovir [98, 99]. Since the toxicities are not overlapping the idea of combination therapy is attractive. Initially a few small uncontrolled series suggested a benefit of combination therapy in patients with recurrent retinitis [100, 101]. The Cytomegalovirus Retinitis Retreatment trial was a large multicenter, randomized controlled trial which addressed the question of whether it is best to treat recurrent retinitis with the high doses of the same drug or a combination of both drugs. In this study patients who had a relapse were either re-induced with the same agent they relapsed on (ganciclovir or foscarnet) or switched to the alternative agent. Maintenance therapy was then given either with one or both drugs. The combination treatment arm was associated with the longest time to progression, 4.3 months versus 1.3 and 2.0 months for foscarnet and ganciclovir monotherapy, respectively. However, combination treatment required the longest infusion time and had the greatest negative impact on quality of life [72]. There was no benefit to switching from one monotherapy arm to the other at the time of recurrence. Thus, based on this study the optimal treatment of recurrence was combination therapy and switching monotherapy was not beneficial for early relapses. Some of the newer therapies may offer advantages over these strategies for the treatment of progression.

The ganciclovir implant has been very successful in the treatment of progression. Marx and colleagues inserted the implant in 91 eyes of 70 patients with recurrent CMV retinitis, who had either failed or were intolerant of ganiclovir or foscarnet. At one month of follow-up 76% of evaluable eyes had inactive retinitis. For those patients with an initial response to treatment the median time to recurrence was 210 days. These data compare favorably to other forms of treatment for recurrence. However, one caveat of the study is that 80% of patients continued to receive systemic treatment during the study. Thus it is difficult to assess the implant alone, although due to the high intravitreal ganciclovir levels achieved by the implant the primary efficacy was probably not related to the systemic therapy. Based on data indicating that the ganciclovir resistance increases with time on treatment, it may be reasonable to treat early recurrences with the implant; however, late recurrences on long term ganciclovir may require an alternative agent, such as cidofovir or foscarnet.

Cidofovir may also be an option for treatment of progression. Lalezari and colleagues randomized 100 patients with progressive CMV retinitis (median of four courses of systemic treatment) and/or intolerance to ganciclovir or foscarnet to intravenous cidofovir 5mg/kg every week for 2 weeks, then either 5mg/kg (49 pts) or 3mg/kg (51 pts) every other week. The median time to progression was 115 days in the high dose arm versus 49 days in the low dose arm; however, side effects due to probenicid were very common [82].

Thus, several options are available for the treatment of progression. The patient can be reinduced and maintained with the same agent, given combination therapy with foscarnet and ganciclovir, can be given local therapy (e.g. implant or intravitreal injections of ganciclovir or foscarnet) or intravenous cidofovir. Optimal therapy for each patient will differ, based on several factors such

as time on prior therapy, zone of retinal involvement, concommitant medications, renal function, and lifestyle.

Prophylaxis

Three randomized, prospective, controlled studies of CMV disease prophylaxis in AIDS patients have been conducted, two with oral ganciclovir and one with valaciclovir, an acyclovir pro-drug. The two studies using oral ganciclovir at a dose of 3000 mg/day in patients with less than 100 CD4 cells reached different conclusions. The first study by Spector et al, found a reduction in rates of CMV disease from 26% to 14% at one year for recipients of oral ganciclivor compared to placebo. Substantially more patients in the ganciclovir group required colony stimulating factors (granulocyte colony stimulating factor and erythropoietin) because of drug related neutropenia [66].

The second study, by Brosgart et al., did not demonstrate a benefit of ganciclovir. However, these studies differed in several important ways. In the study by Brosgart, et al., patients did not undergo screening fundoscopy at entry or subsequently to exclude patients with active CMV retinitis. The CD4 cell counts of participating patients in the study by Brosgart were somewhat lower than in the study by Spector, et al. In addition, the study of Brosgart et al was amended at approximately the midway point, to allow patients assigned to placebo to receive open-label ganciclovir. Nevertheless, no decrease in CMV disease for patients receiving ganciclovir was noted even prior to the open label phase [102]. Neither study demonstrated a survival benefit for ganciclovir treated patients. The study of valaciclovir did demonstrate a decrease in CMV disease, however; because of an unexpected higher mortality in patients receiving valaciclovir, the study was terminated early [103]. Based on equivocal efficacy data and the prohibitive cost of prophylaxis with oral ganciclovir, most clinicians have elected not to routinely prescribe prophylaxis for CMV retinitis until a better agent becomes available.

CMV in the era of HAART

There are several recent reports suggesting that HAART may affect the natural history of CMV retinitis. As mentioned above the incidence of CMV disease has decreased dramatically in the last two years. Times to progression of retinitis may be greatly increased in patients receiving protease inhibitors. In addition, clinical presentations of CMV retinitis may differ due to the effects of immune reconstitution.

Although the use of protease inhibitors has resulted in a decrease in new CMV disease, there are several reports of patients who have been diagnosed with CMV retinitis after the initiation of protease inhibitor therapy, with high CD4 cell counts (>150). In these instances the CD4 nadir was less than 85 cells/ul prior to starting protease inhibitors and CMV disease usually developed within 1-2 months of starting protease therapy [104-107]. In the pre-HAART era it was uncommon to develop CMV retinitis with a CD4 count > 100. In one study from the pre-HAART era only 1.5% of patients were diagnosed with CMV retinitis with a CD4 count above 100 cells/μL [104]. In another study which compared pre and post-HAART era patients with newly diagnosed CMV retinitis, only 4% of pre-HAART patients had a CD4 count over 100 cells/μL , compared to 14% of the HAART era patients [31].

The diagnosis of new CMV retinitis after protease inhibitor initiation suggests one of two possibilities, either CMV retinitis was present but not diagnosed at the onset of protease inhibitor therapy or although HAART results in a rapid increase in CD4 cells, the restoration of the immune system may be incomplete. Although the absolute number of CD4 cells occurs early after initiation of HAART, these cells may not be fully functional and the diversity of the CD4 repertoire may be limited. Another possibility is that the vascular endothelium of the internal compartment of

the eye prevents the passage of cells from the the blood to the eye, which could prevent immune effector cells from reaching the eye [108].

A study by Connors and colleagues provided some support for the incomplete immune reconstitution theory [109]. They measured CD4 cells (naive and memory) in antiretroviral naive patients receiving a protease inhibitor. In patients with moderately low pretreatment CD4 cell counts both memory and naive cells increased over the three month treatment period. In contrast in patients with very low CD4 cell counts there was an increase in memory cells only. They suggested that once the naive cells are destroyed they cannot be regenerated, at least in the first three months.

Another recent study by Autran et al., suggests that naive cells can be regenerated; however, it occurs later, in a second phase of immune recovery [110]. The first phase, which occurs in the first few weeks, results in a redistribution of lymphoid cells (memory and naive cells), which results in rapid increases in the numbers of CD4 and to a lesser extent CD8 lymphocytes. New naive cells (CD4 and CD8) are not produced during this initial phase. In the second phase of immune reconstitution which occurs over one year and possibly beyond, there is a gradual rise in the number of CD4 cells, as new CD4 and CD8 naive cells are made. Autran's data may provide a logical explanation for the apparently contradictory observations of the development of CMV retinitis soon after starting protease inhibitor therapy, in some patients, while on the other hand most patients who respond to HAART have long term protection against CMV. The patient who responds to HAART with an increase in CD4 cell number, may not initially recognize certain antigens, such as CMV, because of loss of CMV specific CD4 cells. Once the individual has the capacity to develop new naive cells which can then recognize new antigens (such as CMV) he may be protected by the development of specific memory cells.

Another unusual finding attributed to HAART has been the development of inflammatory reactions. Prior to the era of immune reconsititution, CMV retinitis was characterized by little or no intraocular inflammation. However, recently both vitritis and macular edema have been described following the start of protease inhibitor therapy [104, 111-113]. Some have suggested that with the immune restoration induced by HAART an inflammatory response may result in unusual manifestations, such as vitritis. Karavellas et al., has coined the term "immune recovery vitritis" to describe the syndrome, which usually responds to topical or oral corticosteroids and is not associated with reactivation of retinitis [113].

The unusual presentations of CMV retinitis recently reported have led some to suggest that patients beginning with low CD4 cells prior to the initiation of HAART be monitored for the development of retinitis even after their CD4 cell increases to levels previously not considered at risk for CMV retinitis [114]. These recommendations seem reasonable until more information is available about the incidence and the specifics of these new presentations.

Screening

By the time CMV retinitis presents there are usually visual symptoms, often with loss of visual acuity, especially with progressive disease. Ideally one goal of CMV retinitis management should be to try to identify patients prior to the onset of symptoms, since current prophylactic therapy is only moderately efficacious and is very expensive.

The utility of routine ophthalmological screening for CMV retinitis in patients with AIDS is debatable [22, 115]. A few studies from the pre-HAART era suggested that in patients with less than 50 CD4 cells unsuspected CMV retinitis occurred in 13% to 19% of individuals [115, 116]. However, a recent study found that only 0.8% of patients with under 100 CD4 cells had previously undiagnosed CMV [117]. The ideal situation would be to use an inexpensive laboratory marker to detect CMV retinitis, thus allowing for identification of patients at highest risk

for the development of CMV retinitis. Targeted prophylaxis could then be used in a cost-effective manner, while minimizing toxicity in patients who are unlikely to benefit.

New Laboratory Markers for CMV

Conventional viral cultures of blood and urine have limited utility in the diagnosis of CMV retinitis. Using these techniques the virus takes several weeks to grow and the results are not quantifiable. The sensitivity of CMV viremia for CMV retinitis or other end-organ disease is relatively low, ranging from 38% to 76% [70, 118-121]. The specificity of viremia for CMV end-organ disease is somewhat better, ranging from 74% to 95% [118-121]. Urine cultures have a higher sensitivity (78% to 100%) but poor specificity (5% to 55%) [70, 118, 119, 122]. Thus CMV blood cultures have poor positive predictive value but somewhat better negative predictive value. Since CMV viremia and viruria are neither sufficiently sensitive nor specific to be clinically useful for the diagnosis of CMV retinitis, other techniques are needed. A more rapid method is the shell vial culture technique, but it is relatively insensitive.

Because of the relatively poor predictive value of blood cultures, investigators have developed other tests to enhance the diagnosis and management of patients with CMV end-organ disease. Most of these tests, such as CMV PCR, DNA blot hybridization, and pp65 antigen test, can give quantitative results, which may correlate with the likelihood of CMV disease [123]. The two tests with the most clinical experience to date are the CMV antigenemia assay and the CMV PCR assay. These tests are potentially useful fo confirm the diagnosis of CMV retinitis in cases where the clinical diagnosis is equivocal, to predict patients at high risk for the developing CMV retinitis, to monitor response to CMV treatment and to predict relapse.

The CMV antigenemia test involves the use of a monoclonal antibody to a CMV specific 65-kd matrix protein (pp65 antigen) in the nuclei of infected neutrophils. A second fluorescein or peroxidase labeled antibody is then used to give a readable result. Results are expressed as the number of positively staining cells per number of cells counted, typically 100,000 to 300,000. Thus the test provides a semiquantitative result. The number of positive cells has been shown to correlate with CMV disease and to predict future development of CMV disease [120, 124-126]. The higher the level of antigenemia, the more likely it is that the patient has clinical disease [127-129]. The test is best used if results are reported quantitatively, since patients with low numbers of positive cells, may not have CMV disease. In one study a low positive result predicted a low likelihood of having active disease (only 26%); however, if a cutoff of 10 CMV positive cells per 100,000 cells counted was used the yield increased significantly to 65% [126]. Some studies have reported that patients with CMV retinitis will have lower levels of positivity compared to patients with gastrointestinal disease [127, 128].

The sensitivity (33% to 97%), and specificity (64% to 100%) of the pp65 antigenemia test vary widely in published reports [102, 120, 125-131]. This may be due to the fact that different methods were used and different populations were studied. In direct comparisons with blood cultures, the antigenemia test has been more sensitive [120, 124, 125, 130]. The antigenemia assay is also useful for monitoring response to CMV therapy and predicting relapse of disease [127-129].

Qualitative CMV PCR is the most sensitive technique for detection of systemic CMV infection; however, the test lacks specificity. Quantitative PCR may be more useful, although it is more cumbersome and the appropriate cutoffs for reporting a positive result remain to be determined. Studies of CMV PCR have amplified DNA from either whole blood, serum or peripheral blood leukocytes. Several studies have shown that increases in CMV DNA levels precede disease as well as relapses, usually by weeks to months [120, 132]. In addition, improvement in CMV disease has been correlated with clearance of CMV DNA from blood [133].

Bowen et al. prospectively followed a group of AIDS patients with < 50 CD4 cells/μL with regular ophthalmologic exams and quantitative CMV PCR. Patients who were initially CMV PCR positive had 20 times the risk of developing CMV disease and for each 0.25 log increase in CMV load the relative hazard for CMV disease was 1.37. The positive predictive value for the development of CMV disease if PCR+ at entry was 0.59. The negative predictive value was excellent; only 3 of 70 patients who were PCR-negative developed CMV disease [134].

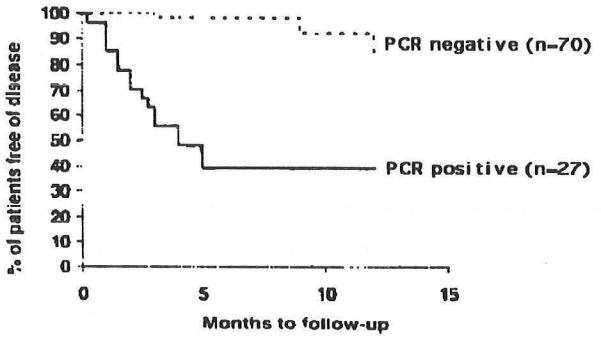


Figure 1. Kaplan-Meier analysis of time to cytomegalovirus disease according to baseline CMV PCR status. (P < 0.0001)
Bowen et al AIDS 1997

In another study the same group of investigators followed 45 pts with CMV retinitis with monthly quantitative CMV PCR of blood and urine. They demonstrated that induction therapy with ganciciclovir was associated with clearance of CMV: 85% of those PCR+ at time of CMV retinitis diagnosis became negative after 21 days of ganciclovir. A higher CMV load at entry correlated with failure to clear CMV from the blood and a trend towards shorter time to progression. Furthermore a high CMV viral load at presentation was independently associated with shorter survival [133].

Shinkai and colleagues followed 94 AIDS patients for a mean of one year and obtained urine and blood cultures as well as qualitative and quantitative PCR, every 3 months. Twenty-eight percent of their cohort developed CMV disease. The qualitative PCR had a sensitivity and specificity of 89% and 75%, respectively. By using quantitative PCR with a cutoff of 100 copies the specificity improved to 90%, while the sensitivity only decreased slightly to 73% [119].

Table 2. Quantitative competitive HCMV plasma PCR results and development of HCMV disease in subjects previously identified to be positive by qualitative plasma DNA PCR.

Peak copies/μL	Disease (n = 24)*	No disease (n = 17)*
>1000†	9 (100)	0
100–1000	10 (59)	7 (41)
<100†	5 (33)	10 (67)
Mean ± SE HCMV copies/μL‡	1510 ± 448	161 ± 52
Median HCMV copies/μL‡	473	35

Shinkai et al. JID 1997

CMV PCR and pp65 antigen testing have only been directly compared in a few studies. Boivin and colleagues reported good correlation for the diagnosis of untreated CMV [131]. In a study of 200 patients with under 100 CD4 cells, Dodt et al. did CMV PCR, pp65 antigen, and CMV blood cultures every 2 months for one year or until development of CMV or death. Thirty-eight patients developed CMV disease after a median of 4.5 months. The PCR, antigen test and CMV blood culture detected CMV disease a median of 46 days, 34 days, and 1 day before disease onset, respectively. The PCR had a sensitivity of 95% and specificity of 85%. The pp65 had a sensitivity of 92% and specificity of 88% and blood culture had a sensitivity of 76% and specificity of 88% [120]. There was also a correlation between a beneficial response to therapy and conversion of PCR or pp65 to negative.

At present no definitive recommendations can be made about which test to use, the CMV PCR or antigenemia assay. The advantages of PCR are that it can can be done on frozen samples, directly measures CMV load and it can be quantified. However, the test has not been well standardized, different methods exist and the quantitative assays are cumbersome. It is not yet available for commercial use. The pp65 test results can be obtained rapidly and is fairly easy to perform. The disadvantages include the requirement for a fluorescent microscope and for fresh blood. In addition, since neutrophils are stained the results may be falsely low in patients with severe

neutropenia. The test has not been well standardized and there is wide intra and interlaboratory variation [135]. The definition of a significant number of positively staining cells varies widely in the literature. Two CMV antigenemia tests are commercially available. The cost ranges from $80 to $320. Ongoing studies should hopefully clarify how best to use and interpret the pp65 antigen test and PCR test in AIDS patients. Ideally, CMV serum markers should predict the development of new CMV disease and relapse with a high degree of sensitivity and specificity, so that patients are not given expensive and potentially toxic therapies, unless they are needed.

In patients with very high CMV DNA levels as measured by either the antigenemia assay or quantitative PCR consideration should be given to preemptive treatment. At the very least these patients should be followed closely for the development of CMV retinitis. Furthermore, the initial CMV viral load could be a factor in determining the length and type of induction therapy.

Sampling of aqueous and vitreous fluid with subsequent PCR on the fluid have also been used for diagnosis, but obviously this is inconvenient to perform on a regular basis [136, 137]. However, in cases of sight threatening retinitis where the diagnosis is unclear, this procedure may be helpful.

Prior to the era of HAART, all patients with CMV retinitis required lifelong maintenance therapy to prevent recurrence. Patients with excellent responses to HAART and CMV therapy may not require lifelong maintenance therapy [104, 138-140]. There are several recent reports suggesting that patients receiving protease inhibitors have substantially longer times to progression [141, 142]. In fact there are sporadic reports of patients with CMV retinitis, who did not receive any CMV specific therapy, who had complete regression of retinitis with HAART alone [112]. In our own preliminary review of 60 CMV retinitis patients treated at the Parkland HIV clinic, those treated with a protease inhibitor had a significantly longer time to progression compared to patients not receiving a protease inhibitor: 751 days versus 312 days [143]. At present it is not known which patients require continued maintenance therapy. In general, the safest strategy is to continue some form of maintenance therapy in all patients until more data becomes available.

Investigational Agents

Several agents for the treatment of CMV retinitis are currently under investigation.
An anti-CMV antibody (MSL-109) to the CMV surface antigen gH was studied as an adjunct to foscarnet or ganciclovir. A small phase I study showed an increased time to progression of over 200 days; however, a larger study failed to demonstrate a benefit of MSL-109 and the group receiving it actually had a higher mortality rate [144, 145].

ISIS-2922 (fomivirsen) is an antisense oligoclonal nucleotide, which is injected intravitreally. It binds to CMV messenger RNA-encoding regulatory proteins and prevents viral replication. It is currently being assessed in clinical trials. It has been associated with peripheral retinopathy, transient increased intraocular pressure and inflammation [146]. Cidofovir is also effective when used intravitreally but has been associated with hypotony and iritis (see above).

Two oral antiviral nucleoside analogues are in phase II-III clinical trials. Lobucavir, a guanine analogue, is active against herpes simplex virus as well as CMV and has good bioavailabity [147]. Valganciclovir, the valine ester of ganciclovir, is rapidly absorbed and converted to ganciclovir. Excellent serum levels are achieved which are equivalent to levels obtained with intravenous ganciclovir [148]. A multicenter phase III randomized comparison of oral valganciclovir versus intravenous ganciclovir for new CMV retinitis is currently underway.

Although the incidence of CMV disease has decreased since the advent of HAART, it is likely that an increasing number of patients will eventually fail these difficult antiretroviral regimens. This may lead to an increase in opportunistic infections such as CMV to levels approaching that of the pre-HAART era. While the future of CMV retinitis in patients with HIV is uncertain, the advances

in diagnosis and management in the past few years have improved the outlook of patients with CMV retinitis. Earlier diagnosis with the aid of rapid virologic techniques should result in prompt treatment and therefore preservation of vision. Newer oral and local agents with fewer toxicities and improved efficacy should make treatment of patients more convenient and more effective.

Bibliograpy

1. CDC: Update: Trends in AIDS Incidence- United States, 1996. MMWR 1997; 46: 861-867.

2. Palella FJ, Delaney KM, Moorman AC, et al: Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998; 338: 853-60.

3. Ho M: History of cytomegalovirus. In: Ho M, ed. Cytomegalovirus: Biology and Infection. New York Plenum Publishing Co., 1991; 1-6.

4. Smith MG: Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. Proc Soc Exp Biol Med 1956; 92: 424-30.

5. Rowe WP, Hartley JW, Waterman S, al. e: Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc Soc Exp Biol Med 1956; 92: 418-24.

6. Weller T, Macauley J, Craig J, Wirth P: Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc Soc Exp Biol Med 1957; 94: 4-12.

7. Christensen L, Beeman HW, Allen A: Cytomegalic inclusion disease. Arch Ophthalmol 1957; 57: 90-9.

8. Foerster HW: Pathology of granulomatous uveitis. Surv Ophthalmol 1959; 4: 296.

9. Murray HW, Knox DL, Green WR, Susel RM: Cytomegalovirus retinitis in adults: a manifestation of disseminated viral infection. Am J Med 1977; 63: 574-582.

10. Moore PS, Chang Y: Detection of herpesvirus-like DNA sequences in Kaposi's Sarcoma in patients with and those without HIV infection. New England Journal of Medicine 1995; 332: 1181-5.

11. Alford CA, Britt WJ: Cytomegalovirus. In: Fields BN, Knipe DM, et al, eds. Virology, 2nd ed, vol 2. New York: Raven Press, Ltd., 1990; 1981-2010.

12. Masur H, Whitcup SM, Cartwright C, Polis M, Nussenblatt R: Advances in the management of AIDS-related cytomegalovirus retinitis. Ann Intern Med 1996; 125: 126-136.

13. Ho M: Cytomegalovirus. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practices of Infectious Diseases, 4th ed, vol 2. New York: Churchill Livingstone Inc., 1995; 1351-1364.

14. Ho M: Epidemiology of cytomegalovirus infection in man. In: Ho M, ed. Cytomegalovirus: Biology and Infection, 2nd ed. New York: Plenum Publishing Co., 1991; 155-188.

15. Onorato IM, Morens DM, Martone WJ, Stansfield SK: Epidemiology of cytomegaloviral infections: recommendations for prevention and control. Rev Infect Dis 1985; 7: 479-97.

16. Collier AC, Meyers JD, Corey L, Murphy VL, Roberts PL, Handsfield HH: Cytomegalovirus infection in homosexual men: relationship to sexual practices, antibody to human immunodeficiency virus, and cell-mediated immunity. Am J Med 1987; 82: 593-601.

17. Mintz L, Drew W, R RM, Braff E: Cytomegalovirus Infections in Homosexual Men. Annals of Internal Medicine 1983; 99: 326-329.

18. Drew WL, Mintz L, Miner RC, Sands M, Ketterer B: Prevalence of cytomegalovirus infection in homosexual men. Journal of Infectious Diseases 1981; 143: 188-192.

19. Gallant JE, Moore RD, Richman DD, Keruly J, Chaisson RE: Incidence and natural history of cytomegalovirus disease in patients with advanced human immunodeficiency virus disease treated with zidovudine. J Infect Dis 1992; 166: 1223-7.

20. Hoover DR, Saah AJ, Bacellar H, et al: Clinical manifestations of AIDS in the era of *Pneumocystis carinii* prophylaxis. N Engl J Med 1993; 329: 1922-26.

21. Drew WL: Cytomegalovirus infection in patients with AIDS. Clin Infect Dis 1992; 14: 608-15.

22. d'Arminio Monforte A, Mainini F, Testa L, et al: Predictors of cytomegalovirus disease, natural history and autopsy findings in a cohort of patients with AIDS. AIDS 1997; 11: 517-24.

23. Michaels S, Clark R, Kissinger P: Differences in the incidence of Opportunistic Processes before and after the availablitiy of protease inhibitors. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, 1998.

24. Murphy R, El-Sadr W, Cheung T, et al: Impact of protease inhibitor containing regimens on the risk of developing opportunistic infections and mortality in the CPCRA 034/ACTG 277 Study. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, 1998.

25. Holtzer CD, Jacobson MA, Hadley WK, et al: Decline in specific opportunistic infections at San Francisco General Hospital. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, 1998.

26. Moore RD, Keruly JC, Chaisson RE: Decline in CMV and other Opportunistic Infections with combination antiretroviral therapy. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, 1998.

27. Baril L, Jouan M, Caumes E, Mengual X, Bricaire F, Katlama C: The Impact of HAART on the incidence of CMV disease in AIDS patients. Abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapeutics, Toronto, 1998.

28. Studies of ocular complications of AIDS research group in collaboration with the AIDS clinical trial group: Foscarnet-Ganciclovir cytomegalovirus retinitis trial: 5. Clinical features of cytomegalovirus retinitis at diagnosis. Am J Opthalmol 1997; 124: 141-57.

29. Saillour F, Bernard N, Dequae-Merchadou L, Marimoutou C, Journot V, Dabis F: Predictive factors of occurrence of cytomegalovirus disease and impact on survival in the Aquitaine cohort in France, 1985 to 1994. Journal of Acquired Immune Deficiency Syndrome 1998; 17: 171-178.

30. d'Arminio Monforte A, Mainini F, Vago L, al e: Factors associated with increased risk for cytomegalovirus disease in AIDS patients. J Infect Dis 1993; 168: 1071-2.

31. Hoover DR, Peng Y, Saah A, et al.: Occurrence of cytomegalovirus retinitis after human immunodeficiency virus immunosuppression. Archives of Ophthalmology 1996; 114: 821-7.

32. Jabs DA: Ocular manifestations of HIV infection. Trans Am Ophthalmol Soc 1995; 93: 623-83.

33. Lowder CY, Butler CP, Dodds EM, Secic M, Recillas-Gispert C: CD8+ T lymphocytes and cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome. American Journal of Ophthalmology 1995; 120: 283-90.

34. Lyles RH, Chu C, Margolick J, et al: HIV RNA and CD4 lymphocyte count relate differently to the occurrence of PCP, CMV, and MAC disease. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, 1998.

35. Currier JS, Williams PL, Grimes JM, Squires KS, Fischl MA, Hammer SM: Incidence rates and risk factors for Opportunistic Infections in a phase III trial comparing indinvavir + ZDV +3TC to ZDV +3TC. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, 1998.

36. Kestelyn P, Van De Perre P, Rouvroy D, et al: A prospective study of the ophthalmic findings in the acquired immune deficiency syndrome in Africa. Am J Ophthalmol 1985; 100: 230-8.

37. Rasmussen L, Morris S, Wolitz R, et al.: Deficiency in antibody response to human cytomegalovirus glycoprotein gH in human immunodeficiency virus-infected patients at risk for cytomegalovirus retinitis. Journal of Infectious Diseases 1994; 170: 673-7.

38. Schrier RD, Freeman WR, Wiley CA, McCutchan JA: Immune predispositions for cytomegalovirus retinitis in AIDS. The HNRC Group. Journal of Clinical Investigation 1995; 95: 1741-6.

39. Park SS, Sigelman J, Gragoudas ES: Anatomy and Cell Biology of the Retina. In: Tasman W, Jaeger EA, eds. Duane's Foundations of Clinical Ophthalmology. Philadelphia: J.B. Lippincott Co., 1994; 1-63.

40. Federman JL, Gouras P, Schubert H, Slusher MM, Vrabec TR: Inflammations of the Retina. In: Podos SM, Yanoff M, eds. Textbook of Ophthalmology, vol 9, Retina and Vitreous. London: Mosby, 1994; 10.1-10.30.

41. Holland G, Gottlieb M, Yee R, Schanker H, Pettit T: Ocular Disorders associated with a new severe acquired cellular immunodeficiency syndrome. American Journal of Ophthalmology 1982; 93: 393-402.

42. Holland GN, Pepose J, Pettit T, Gottlieb M, Yee RD, Foos RY: Acquired Immune Deficiency Syndrome: Ocular Manifestations. Ophthalmology 1983; 90: 859-873.

43. Pepose JS, Holland GN: Cytomegalovirus infections of the retina. In: Ryan SJ, ed. Retina, 2nd ed, vol 2. St. Louis: Mosby-Year Book, Inc., 1994; 1559-1570.

44. Wyhinny G, Apple D, Guastella F, Vygantas C: Adult Cytomegalic inclusion retinitis. American Journal of Ophthalmology 1973; 76: 773-781.

45. Miceli MV, Newsome DA, Novak LC, Beuerman RW: Cytomegalovirus replication in cultured human retinal pigment epithelial cells. Curr Eye Res 1989; 8: 835-9.

46. Rickman LS, Freeman WR: Retinal disease in the HIV infected patient. In: Ryan SJ, ed. Retina, 2nd ed, vol 2. St Louis: Mosby-Yearbook, Inc., 1994; 1571-1596.

47. Apple DJ, Rabb MF: Fundus. In: Apple DJ, Rabb MF, eds. Ocular Pathology: clinical applications and self assessment, 4th ed. St Loius: Mosby Yearbook, 1991; 263-374.

48. Gonzalez CR, Wiley CA, Arevalo JF, et al.: Polymerase chain reaction detection of cytomegalovirus and human immunodeficiency virus-1 in the retina of patients with acquired immune deficiency syndrome with and without cotton-wool spots. Retina 1996; 16: 305-11.

49. Jabs DA, Dunn JP, Enger C, Forman M, Bressler N, Charache P: Cytomegalovirus retinitis and viral resistance. Prevalence of resistance at diagnosis, 1994. Cytomegalovirus Retinitis and Viral Resistance Study Group. Archives of Ophthalmology 1996; 114: 809-14.

50. Bloom JN, Palestine AG: The Diagnosis of cytomegalovirus retinitis. Ann Int Med 1988; 109: 963-69.

51. Batisse D, Eliaszewicz M, Zazoun L, Boudrimont M, Pialous G, Dupont B: Acute retinal necrosis in the course of AIDS: study of 26 cases. AIDS 1996; 10: 55-60.

52. Ormerod LD, Larkin JA, Margo CA, et al: Rapidly progressive herpetic retinitis: A blinding disease characteristic of advanced AIDS. Clin Infect Dis 1998; 26: 34-45.

53. Holland GN: Ocular Sequelae. In: Broder S, Merrigan TC, Bolognesi D, eds. Textbook of AIDS Medicine. Baltimore: Williams and Wilkins, 1994; 473-487.

54. Jacobson MA: Treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome. New England Journal of Medicine 1997; 337: 105-14.

55. Jabs DA, Enger C, Dunn JP, Forman M: Cytomegalovirus retinitis and viral resistance: gancicloivir resistance. J Infect Dis 1998; 177: 770-73.

56. Freeman WR, Friedberg DN, Berry C, et al.: Risk factors for development of rhegmatogenous retinal detachment in patients with cytomegalovirus retinitis. American Journal of Ophthalmology 1993; 116: 713-20.

57. Studies of the Ocular Complications of AIDS Research Group in collaboration with the AIDS Clinical Trials Group: Foscarnet-Ganciclovir cytomegalovirus retinitis trial 4: visual outcomes. Ophthalmology 1994; 101: 1250-61.

58. Jabs DA: Acquired immunodeficiency syndrome and the eye--1996. Archives of Ophthalmology 1996; 114: 863-6.

59. Drew WL, Ives D, Lalezari JP, et al.: Oral ganciclovir as maintenance treatment for cytomegalovirus retinitis in patients with AIDS. Syntex Cooperative Oral Ganciclovir Study Group [see comments]. New England Journal of Medicine 1995; 333: 615-20.

60. Crumpacker CS: Ganciclovir. N Engl J Med 1996; 335: 721-729.

61. Spector SA, Busch DF, Follansbee S, et al: Pharmacokinetics, safety, and antiviral profiles of oral ganciclovir in persons infected with human immunodeficiency virus: a phase I/II study. J Infect Dis 1995; 171: 1431-7.

62. Plotkin SA, Drew WL, Felsentsein D, Hirsch MS: Sensitivity of clinical isolates of human cytomegalovirus to 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine. J Infect Dis 1985; 152: 833-34.

63. Arevalo JF, Gonzalez C, Capparelli EV, et al.: Intravitreous and plasma concentrations of ganciclovir and foscarnet after intravenous therapy in patients with AIDS and cytomegalovirus retinitis. Journal of Infectious Diseases 1995; 172: 951-6.

64. Lalezari J, Friedberg D, Bisset J, Giordano J, Hardy D, Robinson C: A Comparison of the safety and efficacy of 3G, 4.5G and 6G doses of oral ganciclovir versus IV ganciclovir for maintenance treatment of CMV retinitis. 11th International Conference on AIDS, Vancouver, BC, 1996.

65. The Oral Ganciclovir European and Australian Cooperative Study Group: Intravenous versus oral ganciclovir: European/Australian comparative study of efficacy and safety in the prevention of cytomegalovirus retinitis recurrence in patients with AIDS. AIDS 1995; 9: 471-7.

66. Spector SA, McKinley GF, Lalezari JP, et al: Oral gancicloivir for the prevention of cytomegalovirus disease in persons with AIDS. N Engl J Med 1996; 334: 1491-97.

67. Dunn JP, MacCumber MW, Forman MS, Charache P, Apuzzo L, Jabs DA: Viral sensitivity testing in patients with cytomegalovirus retinitis clinically resistant to foscarnet or ganciclovir. American Journal of Ophthalmology 1995; 119: 587-96.

68. Jabs DA, Dunn JP, Enger C, Forman M, Bressler N, Charache P: Cytomegalovirus retinitis and viral resistance. Prevalence of resistance at diagnosis, 1994. Cytomegalovirus Retinitis and Viral Resistance Study Group. Arch Ophthalmol 1996; 114: 809-14.

69. Holland GN, Levinson RD, Jacobson MA: Dose-related difference in progression rates of cytomegalovirus retinopathy during foscarnet maintenance therapy. AIDS Clinical Trials Group Protocol 915 Team. American Journal of Ophthalmology 1995; 119: 576-86.

70. Palestine AG, Polis MA, DeSmet MD, et al: A Randomized, controlled trial of Foscarnet in the treatment of cytomegalovirus retinitis in patients with AIDS. Ann Intern Med 1991; 115: 665-73.

71. Studies of Ocular Complications of AIDS Research Group: Mortality in Patients With The Acquired Immunodeficiency Syndrome Treated With Either Foscarnet or Ganciclovir for Cytomegalovirus Retinitis. N Engl J Med 1992; 326: 213-20.

72. Studies of Ocular Complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group: Combination foscarnet and ganciclovir therapy vs monotherapy for the treatment of relapsed cytomegalovirus retinitis in patients with AIDS: The Cytomegalovirus Retreatment Trial. Arch Ophthalmology 1996; 114: 23-33.

73. Studies of ocular complications of AIDS Research Group in collaboration with the AIDS Clinical Trials Group: Morbidity and toxic effects associated with ganciclovir or foscarnet therapy in a randomized cytomegalovirus retinitis trial. Arch Intern Med 1995; 155: 65-74.

74. Polis MA, DeSmet MD, Baird BF, et al: Increased survival of a cohort of patients with acquired immunodeficiency syndrome and cytomegalovirus retinitis who received sodium phosphonoformate (Foscarnet). Am J Med 1993; 94: 175-180.

75. Drew WL, Miner RC, Busch DF, et al.: Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. J Infect Dis 1991; 163: 716-9.

76. Smith IL, Cherrington JM, Jiles RE, Fuller MD, Freeman WR, Spector SA: High-level resistance of cytomegalovirus to ganciclovir is associated with alterations in both the UL97 and DNA polymerase genes. J Infect Dis 1997; 175: 69-77.

77. Gilbert C, Handfield J, Toma E, Lalonde R, Bergeron MG, Boivin G: Emergence and prevalence of cytomegalovirus UL97 mutations associated with ganciclovir resistance in AIDS patients. AIDS 1998; 12: 125-29.

78. Jabs DA: Incidence of resistant cytomegalovirus (CMV) in patients being treated for CMV retinitis. Abstracts of the 5th Conference on retroviruses and oppportunistic infections, Chicago, IL, 1998.

79. Hitchcock MJ, Jaffe HS, Martin JC, Stagg RJ: Cidofovir, a new agent with potent anti-herpesvirus activity. Antiviral Chemistry & Chemotherapy 1996; 7: 115-27.

80. Lalezari JP, Stagg RJ, Kuppermann BD, et al.: Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS. A randomized, controlled trial. Annals of Internal Medicine 1997; 126: 257-63.

81. Studies of Ocular complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group: Parenteral cidofovir for cytomegalovirus retinitis in patients with AIDS: the HPMPC peripheral cytomegalovirus retinitis trial. A randomized, controlled trial. Annals of Internal Medicine 1997; 126: 264-74.

82. Lalezari JP, Kemper C, Stagg RJ: A randomized, controlled study of the safety and efficacy of intravenous cidofovir (CDV,HPMPC) for the treatment of relapsing cytomegalovirus retinitis in patients with AIDS. Abstracts of the XI International Conference on AIDS, Vancouver, B.C., 1996.

83. Lalezari JP, Drew WL, Glutzer E, et al: (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (cidofovir): Results of a phase I/II study of a novel antiviral nucleotide analogue. J Infect Dis 1995; 171: 88-96.

84. Kirsch LS, Arevalo JF, De Clercq E, et al.: Phase I/II study of intravitreal cidofovir for the treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome. American Journal of Ophthalmology 1995; 119: 466-76.

85. Davis JL, Takintuna I, Freeman WR: Iritis and hypotony after treatment with intravenous cidofovir for cytomegalovirus retinitis. Arch Ophthalmol 1997; 115: 733-37.

86. Cochereau-Massin I, Lehoang P, Lautier-Fraue M, et al: Efficacy and tolerance of intravitreal ganciclovir in cytomegalovirus retinitis in acquired immunodeficiency syndrome. Ophthalmology 1991; 98: 1348-55.

87. Diaz-Llopis M, Espana E, Munoz G, et al: High dose intravitreal foscarnet in the treatment of cytomegalovirus retinitis in AIDS. Br J Ophthalmol 1994; 78: 120-24.

88. Engstrom RE, Jr., Holland GN: Local therapy for cytomegalovirus retinopathy. American Journal of Ophthalmology 1995; 120: 376-85.

89. Hodge WG, Lalonde RG, Sampalis J, Deschenes J: Once-weekly intraocular injections of ganciclovir for maintenance therapy of cytomegalovirus retinitis: clinical and ocular outcome. Journal of Infectious Diseases 1996; 174: 393-6.

90. Martin DF, Parks DJ, Mellow SD, et al.: Treatment of cytomegalovirus retinitis with an intraocular sustained-release ganciclovir implant. A randomized controlled clinical trial. Archives of Ophthalmology 1994; 112: 1531-9.

91. Martin DF, Ferris FL, Parks DJ, et al.: Ganciclovir implant exchange. Timing, surgical procedure, and complications. Archives of Ophthalmology 1997; 115: 1389-94.

92. Musch DC, Martin DF, Gordon JF, Davis MD, Kuppermann BD: Treatment of cytomegalovirus retinitis with a sustained-release ganciclovir implant. The Ganciclovir Implant Study Group. New England Journal of Medicine 1997; 337: 83-90.

93. Martin D, Kuppermann B, Wolitz R, et al: Combined Oral Ganciclovir (GCV) and intravitreal GCV implant for treatment of patients with cytomegalovirus (CMV) retinitis: a randomized, controlled study. 37th Interscience Conference on Antimicrobial Agents and Chemotherapeutics, Toronto, 1997.

94. Kirsch LS, Arevalo JF, Chavez de la Paz E, Munguia D, de Clercq E, Freeman WR: Intravitreal cidofovir (HPMPC) treatment of cytomegalovirus retinitis in patients with acquired immune deficiency syndrome. Ophthalmology 1995; 102: 533-42.

95. Chavez-de la Paz E, Arevalo JF, Kirsch LS, et al.: Anterior nongranulomatous uveitis after intravitreal HPMPC (cidofovir) for the treatment of cytomegalovirus retinitis. Analysis and prevention. Ophthalmology 1997; 104: 539-44.

96. Rahhal FM, Arevalo JF, Chavez de la Paz E, Munguia D, Azen SP, Freeman WR: Treatment of cytomegalovirus retinitis with Intravitreal cidofovir in patients with AIDS. Ann Intern Med 1996; 125: 98-103.

97. Rahhal FM, Arevalo JF, Munguia D, et al.: Intravitreal cidofovir for the maintenance treatment of cytomegalovirus retinitis. Ophthalmology 1996; 103: 1078-1083.

98. Manischewitz JF, Quinnan GV, Lane HC, Wittek AE: Synergistic effect of ganciclovir and foscarnet on cytomegalovirus replication in vitro. Antimcrob Agents Chemother 1990; 34: 373-75.

99. Freitas VR, Fraser-Smith EB, Matthews TR: Increased efficacy of ganciclovir in combination with foscarnet against cytomegalovirus and herpes simplex virus type 2 in vitro and in vivo. Antiviral Research 1989; 12: 205-12.

100. Weinberg DV, Murphy R, Naughton K: Combined daily therapy with intravenous ganciclovir and foscarnet for patients with recurrent cytomegalovirus retinitis. American Journal of Ophthalmology 1994; 117: 776-82.

101. Kuppermann BD, Flores-Aguilar M, Quiceno JI, Rickman LS, Freeman WR: Combination ganciclovir and foscarnet in the treatment of clinically resistant cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome. Arch Ophthalmol 1993; 111: 1359-66.

102. Brosgart CL, Louis TA, Hillman DW, et al: A randomized, placebo-controlled trial of the safety and efficacy of oral ganciclovir for prophylaxis of cytomegalovirus retinitis in HIV-infected individuals. AIDS 1998; 12: 269-277.

103. Feinberg J, Hurwitz S, Cooper D, et al: A Randomized, double-blind trial of valaciclovir prophylaxis for cytomegalovirus disease in patients with advanced human immunodeficiency virus infection. J Infect Dis 1998; 177: 48-56.

104. Jacobson MA, Zegans M, Pavan PR, et al.: Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet 1997; 349: 1443-5.

105. Mallolas J, Arrizabalaga J, Lonca M, et al: Cytomegalovirus disease in HIV-1 infected patients treated with protease inhibitors. AIDS 1997; 11: 1785-87.

106. Gilquin J, Piketty C, Thomas V, et al: Acute CMV infection in AIDS patients receiving combination therapy including protease inhibitors. Abstracts of the 4th Conference on Retroviruses and Opportuntistic Infections, Washington, DC, 1997.

107. Michelet C, Allavena C, Arvieux C, et al: Opportunistic infection occurred under protease inhibitors in HIV patients. 37th Interscience Conference on Antimicrobial Agents and Chemotherapeutics, Toronto, 1997.

108. Streilein JW: Regional immunology of the eye. In: Pepose JS, Holland GN, Wilhelmus KR, eds. Ocular infection and immunology. St. Louis: Mosby, 1996; 19-32.

109. Connors M, Kovacs JA, Krevat S, et al: HIV infection induces changes in CD4 T-cell phenotype and depletions within the CD4 T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. Nature Medicine 1997; 3: 533-40.

110. Autran B, Carcelain G, Li S, et al: Positive effects of combined antiretroviral therapy on CD4 T cell homeostasis and function in advanced HIV disease. Science 1997; 277: 112-116.

111. Stein DS: Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet 1997; 350: 589-90.

112. Reed J, Schwab I, Gordon J, Morse L: Regression of cytomegalovirus retinitis associated with protease-inhibitor treatment in patients with AIDS. Am J Ophthalm 1997; 124: 199-205.

113. Karavellas M, Lowder C, Macdonald J, Avila C, Freeman W: Immune recovery vitritis associated with inactive cytomegalovirus retinitis. Archives of Ophthalmology 1998; 116: 169-75.

114. Ives DV: Cytomegalovirus disease in AIDS. AIDS 1997; 11: 1791-97.

115. Baldassano V, Dunn JP, Feinberg J, Jabs DA: Cytomegalovirus retinitis and low CD4+ T-lymphocyte counts. N Engl J Med 1995; 333: 670.

116. Kuppermann BD, Petty JG, Richman DD: Correlation between CD4 counts and prevalence of cytomegalovirus retinitis and human immunodeficiency virus-related noninfectious retinal vasculopathy in patients with acquired immunodeficiency syndrome. Am J Ophthalmol 1993; 115: 575-82.

117. Brosgart C, Pulling D, Chaloner K, et al: Prevalence of asymptomatic CMV retinitis in AIDS patients. Abstracts of the 5th Conference on retroviruses and oppportunistic infections, Chicago, IL, 1998.

118. Zurlo JJ, O'Neill D, Polis MA, et al: Lack of clinical utility of cytomegalovirus blood and urine cultures in patients with HIV infection. Ann Intern Med 1993; 118: 12-7.

119. Shinkai M, Bozette SA, Powderly W, Frame P, Spector SA: Utility of urine and leukocyte cultures and plasma DNA polymerase chain reaction for idenification of AIDS patients at risk for developing human cytomegalovirus disease. J Infect Dis 1997; 175: 302-8.

120. Dodt KK, Jacobsen PH, Hoffman B, et al: Development of CMV disease may be predicted in HIV-infected patients by CMV polymerase chain reaction and the antigenemia test. AIDS 1997; 11: F21-F28.

121. Gerard L, Leport C, Flandre P, et al: Cytomegalovirus (CMV) viremia and the CD4 lymphocyte count as predictors of CMV disease in patients with human immunodeficiency virus. Clin Infect Dis 1997; 24: 836-40.

122. MacGregor RR, Pakola SJ, Graziani AL, et al.: Evidence of active cytomegalovirus infection in clinically stable HIV-infected individuals with CD4+ lymphocyte counts below 100/microliters of blood: features and relation to risk of subsequent CMV retinitis. Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology 1995; 10: 324-30.

123. Saltzman RL, Quirk MR, Jordan MC: High levels of circulating cytomegalovirus DNA reflect visceral organ disease in viremic immunosuppressed patients other than marrow recipients. J Clin Invest 1992; 90: 1832-38.

124. Landry ML, Ferguson D: Comparison of quantitative cytomegalovirus antigenemia assay with culture methods and correlation with clinical disease. J Clin Microbiol 1993; 31: 2851-56.

125. Wetherill PE, Landry ML, Alcabes P, Friedland G: Use of a quantitative cytomegalovirus (CMV) antigenemia test in evaluating HIV+ patients with and without CMV disease. J AIDS 1996; 12: 33-37.

126. Podzamczer D, Ferrer E, Garcia A, et al.: pp65 antigenemia as a marker of future CMV disease and mortality in HIV-infected patients. Scandinavian Journal of Infectious Diseases 1997; 29: 223-7.

127. Salzberger B, Franzen C, Fatkenheuer G, et al: CMV-antigenemia in peripheral blood for the diagnosis of CMV disease in HIV-infected patients. J AIDS 1996; 11: 365-369.

128. Bek B, Boeckh M, Lepenies J, al e: High-level sensitivity of quantitative pp65 cytomegalovirus (CMV) antigemia assay for diagnosis of CMV disease in AIDS patients and follow-up. J Clin Microbiology 1996; 34: 457-59.

129. Francisci D, Tosti A, Preziosi R, et al: Role of antigenemia assay in the early diagnosis and prediction of human cytomegalovirus organ involvment in AIDS patients. Eur J Clin Microbiol Infect Dis 1995; 14: 498-503.

130. Pannuti CS, Kallas EG, Muccioli C, et al.: Cytomegalovirus antigenemia in acquired immunodeficiency syndrome patients with untreated cytomegalovirus retinitis. American Journal of Ophthalmology 1996; 122: 847-52.

131. Boivin G, Gilbert C, Morissette M, Handfield J, Goyette N, Bergeron MG: A case of ganciclovir-resistant cytomegalovirus (CMV) retinitis in a patient with AIDS: longitudinal

molecular analysis of the CMV viral load and viral mutations in blood compartments. Aids 1997; 11: 867-73.

132. Drouet E, Boibieux A, Michelson S, al. e: Polymerase chain reaction detection of cytomegalovirus DNA in peripheral blood leukocytes as a predictor of cytomegalovirus disease in HIV-infected patients. AIDS 1993; 7: 665-68.

133. Bowen EF, Wilson P, Cope A, et al.: Cytomegalovirus retinitis in AIDS patients: influence of cytomegaloviral load on response to ganciclovir, time to recurrence and survival. Aids 1996; 10: 1515-20.

134. Bowen EF, Sabin CA, Wilson P, et al.: Cytomegalovirus (CMV) viraemia detected by polymerase chain reaction identifies a group of HIV-positive patients at high risk of CMV disease. Aids 1997; 11: 889-93.

135. The TH, van den Berg AP, Harmsen MC, van der Bij W, van Son WJ: The cytomegalovirus antigenemia assay: A plea for standardization. Scan J Infect Dis Suppl 1995; 99: 25-29.

136. Danise A, Cinque P, Vergani S, et al: Use of polymerase chain reaction assays of aqueous humor in the differential diagnosis of retinitis in patients infected with human immunodeficiency virus. Clin Infect Dis 1997; 24: 1100-6.

137. Doornenbal P, Seerp Baarsma G, Quint WG, Kijlstra A, Rothbarth PH, Niesters HG: Diagnostic assays in cytomegalovirus retinitis: detection of herpesvirus by simultaneous application of the polymerase chain reaction and local antibody analysis on ocular fluid. British Journal of Ophthalmology 1996; 80: 235-40.

138. Whitcup SM, Fortin E, Nussenblatt RB, Polis MA, Muccioli C, Belfort R, Jr.: Therapeutic effect of combination antiretroviral therapy on cytomegalovirus retinitis. JAMA 1997; 277: 1519-20.

139. Uthayakumar S, Birthistle K, Dalton R, Hay PE: Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet 1997; 350: 588-9; discussion 590.

140. Jacobson MA: Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy (letter). Lancet 1997; 350: 590.

141. Losso M, Luppo S, Cassetti I, et al: The Effect of protease inhibitors comcommitant therapy on the efficacy of oral ganciclovir for the maintenance treatment of CMV retinitis. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, 1998.

142. Gerard M, Postelmans L, DeWit S, et al: Impact of protease inhibitors (PI) on the incidence of recurrent CMV retinitis (CMVR). Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, 1998.

143. Chiller T, Park A, Chiller K, Skiest D, Keiser P: Protease inhbitor therapy is associated with longer time to relapse in patients with CMV retinitis. 12th World AIDS Conference, Geneva, 1998.

144. Studies of ocular complications of AIDS Research Group in collaboration with the AIDS Clinical Trials Group: MSL-109 adjuvant therapy for cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome: the Monoclonal antibody cytomegalovirus retinitis trial. Arch Ophthalmol 1997; 104: 1528-36.

145. Tolpin M, Pollard R, Tierney M, et al: Combination therapy of cytomegalovirus (CMV) retinitis with a human monclonal anti-CMV antibody (SDZ MSL-109) and either ganciclovir or foscarnet. IX International Conference on AIDS, Berlin, 1993.

146. Muccioli C, Goldstein DA, Johnson DW, et al: Fomvirsen safety and efficacy in the treatment of CMV retinitis: A phase 3, controlled multicenter study comparing immediate versus delayed treatment. Abstracts of the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, 1998.

147. Lalezari JP, Drew WL, Jordan C, et al: In vivo anti-CMV activity and safety of oral lobucavir in HIV-infected patients. Abstracts of the 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997.

148. Brown F, Arum I, Francis G, et al: Ganciclovir prodrug (RS-79070), multiple dose, dose-ranging study with effect of food. Abstracts of the 4th Conference on Retroviruses and Opportunistic Infections, Washington, D.C., 1997.