Antiviral Therapy of CMV Disease in Children

Mike Sharland, Suzanne Luck, Paul Griffiths, and Mark Cotton

Abstract Cytomegalovirus (CMV) remains an important cause of morbidity and mortality in infants and children. The main burden of disease occurs in congenital infection, postnatal infection in premature infants and in older immunocompromised children (now predominantly following transplantation) in developed countries. In lower income countries, CMV is a major co-pathogen in human immunodeficiency virus [HIV]-infected infants. Antiviral treatment options remain very limited. The guanosine analogue ganciclovir (GCV) was first used in children over 20 years ago, but the optimal dose, duration and route of administration remain poorly evidence based. In particular there are very limited data in premature infants and older children. Direct comparison studies between the intravenous ganciclovir and the oral valyl-ester valganciclovir (VGCV) have not been performed. CMV disease is important, but not very common and there remains a need to identify useful surrogate markers of successful antiviral therapy to facilitate clinical trials. Cidofovir and foscarnet have very significant toxicity. No other anti-CMV agent has successfully completed phase III studies. There remain few other antiviral agents effective against CMV on the horizon. This chapter reviews the current clinical spectrum of CMV disease in childhood and the evidence base for both GCV and VGCV use in clinical practice. It also discusses the antiviral studies currently being performed and those that need to be performed.

1 Spectrum of Clinical Disease

1.1 Congenital CMV

Congenital CMV (cCMV) is the most common congenital infection in developed countries, causing very significant long-term disability due to deafness and mental handicap [1, 2]. Birth prevalence varies widely both between and within different

M. Sharland (✉)
Paediatric Infectious Diseases Unit, St. George's Hospital, London, UK
e-mail: mike.sharland@stgeorges.nhs.uk

N. Curtis et al. (eds.), *Hot Topics in Infection and Immunity in Children VII*,
Advances in Experimental Medicine and Biology 697, DOI 10.1007/978-1-4419-7185-2_17,

countries and has been reported to occur in around 0.18–6.2% of live births [2]. A study based in London reported CMV infection in 0.33% of live births [3]. Similar prevalence has been reported in Austria and Italy [2]. Historically the diagnosis has been made by isolation of CMV from a body fluid within the first 21 days of an infant's life. The development of methods for detecting CMV DNA in blood captured on Guthrie cards (dried heel-prick blood spots) taken after birth in most developed countries has enabled the retrospective diagnosis of congenital CMV in older babies, although not all infected babies have detectable viraemia at birth. CMV disease also occurred commonly in infants born to mothers with HIV and CMV coinfection prior to the use of perinatal antiretroviral therapy and the subsequent marked reduction in mother to child transmission of HIV. This remains a major problem in resource-poor countries.

1.1.1 Clinical Features

Congenital CMV infection is usually asymptomatic. A recent meta-analysis reported that symptomatic infection (historically referred to as CMV inclusion disease or CID) is seen in around 12.7% (0.0–25%) of babies with cCMV [2]. The classical features of CID are thrombocytopenia, blueberry muffin rash, petechiae, intrauterine growth retardation (IUGR), microcephaly, hepatosplenomegaly and jaundice (Table 1). Long-term sequelae are mainly in the form of sensorineural hearing loss, neurodevelopmental delay and, rarely, visual loss.

Table 1 Definition of symptomatic disease

One or more of the following
a. Thrombocytopenia
b. Petechiae
c. Hepatomegaly
d. Splenomegaly
e. Intrauterine growth restriction
f. Hepatitis (elevated transaminases and/or bilirubin)
g. Central nervous system involvement of the CMV disease [including microcephaly, radiographic abnormalities indicative of CMV CNS disease, abnormal CSF indices for age, chorioretinitis, hearing deficits as detected by formal brainstem-evoked response (not a screening auditory brainstem response ABR) and/or positive CMV PCR from CSF]

Studies have reported that 40–58% of infants who are symptomatic at birth develop permanent sequelae compared to 13.5% of those without symptoms [1]; around 18% of all congenitally infected infants will be left with some long-term impairment. Characteristic of CMV has been the discovery that hearing loss may be progressive, fluctuate and have a late onset. The pathological processes underlying this delayed presentation of symptoms are currently uncertain and the true incidence is probably underestimated due to inadequate follow-up periods [1]. Overall congenital CMV is reported to cause around 20% of all cases of sensorineural hearing loss and up to 40% of profound hearing loss cases in childhood [4, 5]. CMV

therefore has very significant cost implications for the wider community relating to the healthcare needs, education and support of these children.

1.1.2 Predicting Long-Term Neurological Impairment

Clinical Associations

IUGR, petechiae, hepatosplenomegaly, hepatitis, thrombocytopenia and intracerebral calcifications have been established as risk factors for subsequent hearing impairment in one cohort study, although only IUGR and petechiae remained independent risk factors following multiple regression analysis [6]. Another study has reported microcephaly (adjusting head (occipito-frontal) circumference for weight) as being the most specific predictor for poor neurological outcome whereas a normal CT scan and head circumference were associated with a good outcome [7]. However, clinical features alone are of limited use in predicting those destined for subsequent sensorineural hearing loss, particularly if babies are only mildly symptomatic or asymptomatic at birth. There remains a real need to develop surrogate markers for prediction of long-term outcomes in clinical trials.

Viral Load

Studies in adult transplant recipients have correlated systemic viral load with the risk of clinical disease [8, 9]. An early culture-based study of serial urine specimens taken from both congenitally infected and postnatally infected infants conducted over 30 years ago showed that viral load in the early neonatal period was higher in those babies with symptomatic congenital infection when compared to those with asymptomatic or postnatal infection [10]. The presence of viraemia was first reported to be associated with a poor hearing outcome in neonates with CMV disease in 2005 [11], and subsequently an association has been reported between quantitative CMV viral load at birth in urine, blood and in dried blood spots and symptomatic infection later in childhood [6, 12–14]. Recent data from one of the groups that initially described an association between viral load and hearing outcome do not demonstrate blood viral load to be different between those with and without subsequent hearing loss among either symptomatic or asymptomatic babies. Despite this, among asymptomatic babies, a low blood viral load continued to have a good negative predictive value for an abnormal hearing outcome [15].

A few small studies have reported on virus detection in CSF [16, 17]. In one of these studies only babies with symptomatic CMV had virus detectable, although only at low levels (<400 copies/mL). Based on experience gained with neonatal herpes simplex virus infection, the measurement of CMV in CSF is being carried out by some clinicians as a marker for disease progression and a guide for commencing treatment. Formal studies regarding the utility of carrying out such procedures diagnostically are therefore needed and may help to inform treatment in asymptomatic or mildly symptomatic babies.

1.2 PostNatal CMV

Infection in the neonatal period is most commonly acquired from ingestion of CMV-infected maternal breast milk. The risk of CMV transmission via breast milk was first identified in the late 1960s and in term babies, infection was nearly always found to be asymptomatic [18]. However, using polymerase chain reaction (PCR) to detect CMV in breast milk, up to 96% of seropositive women have been found to have CMV DNA detectable in their breast milk, termed DNAlactia. Some authors have reported transmission in up to 37% of premature babies of whom a significant proportion then develops symptoms [19, 20]. Not all authors have found such high rates of transmission, and a summary of studies addressing the issue of transmission via breast milk can be found in a recent review [21]. The different rates of transmission and symptomatic postnatal disease reported across Europe in different studies may well be in part due to variation in feeding practices in different centres.

1.2.1 Clinical Features

A number of clinical symptoms and signs have been described in association with symptomatic postnatal CMV infection including pneumonitis, hepatosplenomegaly, lymphadenopathy, enteritis and aseptic meningitis [22, 23]. Hamprecht et al. described a sepsis-like syndrome in 4/16 infants with symptoms associated closely with the onset of CMV detection in blood and urine [20]. Other studies have since confirmed this finding, a summary of which can be found in a recent review [21].

Data are very limited on long-term follow-up of infants with postnatal acquisition of CMV. Initial studies of term infants showed no obvious long-term sequelae. Some concerns were raised when a study in preterm infants indicated a trend towards neurological impairment in infants <2,000 g who were infected postnatally [24]. More recent studies, including a prospective, matched, case–control study, do not support this finding [25].

Data reporting CMV viral load for babies treated for postnatally acquired CMV disease are confined to single case reports. How viral load correlates with symptoms and response to treatment remains completely undefined in this age group. The current standard of care for the treatment of premature infants with symptomatic CMV disease is 2 weeks of IV GCV at 12 mg/kg/day.

1.3 CMV Disease in Older Children

CMV in older children is predominantly a disease of the immunocompromised. The main burden of CMV disease in Europe is currently in children undergoing either haemotopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT), predominantly renal, liver, lung or heart. Using quantitative PCR, it can be shown that CMV replicates rapidly in vivo. The replication dynamics of primary CMV infection in adult allograft recipients are very similar to those of primary HIV infection [26]. The dynamics of CMV replication is very rapid in these

patients [8] and multivariable statistical analysis shows that measures of viral load explain all of the previously recognised risk factors for CMV end-organ disease [9, 27, 28]. The serial measurement of viral load in post-transplant patients can be used to guide initiation and cessation of antiviral therapy and to monitor for the emergence of antiviral resistance. Such pre-emptive therapy is now widely used for the management of both adults and children undergoing transplantation across Europe, although paediatric clinical practice is largely based on adult data.

CMV infection has also long been recognised as a cause of serious pneumonitis, retinitis and organ disease in children with HIV in Europe and the USA. Since the introduction of combination antiretroviral therapy, CMV disease is now rare in this setting. However, CMV continues to be a very serious cause of disease in children born with HIV in the resource-poor setting. CMV has clearly been linked to rapidly progressive HIV in young South African infants, often associated with *Pneumocystis* pneumonitis [29]. In 2003, 24 of 47 infants admitted with pneumonitis had evidence of CMV infection [30]. Similarly, CMV was implicated in 50% of HIV-infected infants < 1 year of age not responding to antibiotics within 48 hours in Kwazulu-Natal in the pre-antiretroviral era [31]. CMV retinitis has also been described in HIV-infected infants and children in Cape Town. O'Connell and colleagues described six children (mean age 6.7 months) with CMV retinitis [32]. Currently as antenatal testing for HIV is not universal in high-prevalence African countries, many children are being born with both HIV and CMV disease. However, there is now widespread implementation of combination antiretroviral therapy and in some centres children are also being treated for CMV disease. The relative risks and benefits of IV GCV and oral VGCV in this setting also remain important questions.

2 Ganciclovir and Valganciclovir Use in Children

2.1 Pharmacokinetics

2.1.1 Ganciclovir

GCV is a deoxyguanosine analogue that is triphosphorylated intracellularly to its active metabolite. The CMV encoded UL97 protein kinase is necessary for the first phosphorylation step. The triphosphate then acts as an alternate substrate with the other deoxynucleoside triphosphates, ultimately leading to viral DNA chain termination. The drug is excreted virtually unchanged by the kidneys, with minimal protein binding, but oral absorption is poor. The use of GCV in children was first reported in the late 1980s with most early reports involving children coinfected with HIV. Over the subsequent two decades there have been very few further studies reporting pharmacokinetic data in the paediatric age group and even fewer involving neonates who are well known to have distinct pharmacokinetics. There is a dearth of knowledge from controlled or randomised clinical trials, and interpretation of studies conducted so far is hampered by the difficulty in comparing results

using different drug doses, duration of therapy or follow-up period, as well as different study end points. In neonates, plasma levels comparable to those found in adults can be achieved with intravenous (IV) dosing of 6 mg/kg/dose twice daily (Table 2).

Table 2 Pharmacokinetic parameters of ganciclovir in neonates

Age	Single dose[a]	Cl (L/h/kg)	Vd (L/kg)	C_{max} (μg/mL)	$t_{1/2}$ (h)	References	Number of cases
4–49 days	4 mg/kg	0.19 ± 0.03	0.67 ± 0.07	5.5 ± 0.4	2.4 ± 0.4	Trang et al. [33]	14
2–30 days	6 mg/kg	0.21 ± 0.02	0.75 ± 0.07	7.0 ± 0.5	2.4 ± 0.4	Trang et al. [33]	13
NA	4–6 mg/kg	0.42 ± 0.08	1.8 ± 0.32	NA	NA	Zhou et al. [34]	27
Adult	NA	0.28 ± 0.11	1.1 ± 0.30	6.6 ± 0.8b	4.3 ± 1.6	Pacifici et al. [35]	NA

Adapted from Pacifici et al. [35]. The figures are the mean ± SEM.
[a]Ganciclovir was administered by an intravenous infusion over 1 h.
[b]Following a single 6 mg/kg dose over 1 h of infusion.
Reproduced from Expert Rev. Anti-infect. Ther. 4(6), 1005–1020 (2006) with permission of Expert Reviews Ltd. [36].

Changes in body weight and age have been correlated with changes in the volume of distribution and creatinine clearance of GCV, respectively [33, 34]. A recent pharmacokinetic study in a group of congenitally infected infants aged 30 days or less at the start of treatment showed an increase of 73% in renal excretion of GCV from 3.8 mL/min/kg on day 4 to 6.8 mL/min/kg on day 34 following a dose of IV GCV; a corresponding decrease in the area under the curve (AUC) of 41% over the same time period was observed [37]. This has potential implications for dosing regimens during prolonged periods of GCV treatment used in the period of rapid postnatal growth seen in neonates.

Even in older children, very limited GCV pharmacokinetic (PK) studies have been performed, usually in children treated for CMV after renal transplantation. After a first report in three children [38], the main study from the same group reported on a further 11 children with a mean age of 11 years [39] and the dose of 5 mg/kg used in this study has now been widely adopted, based on very limited data. Another study has subsequently reported sub-therapeutic trough levels (<0.5 mg/L) in 8/9 children treated with this dose of IV GCV, as well as wide inter- and intra-patient variability [40].

2.1.2 Valganciclovir

VGCV is the L-valyl-ester pro-drug of GCV and after oral administration it is rapidly converted to GCV by hepatic and gastrointestinal esterases. Unlike GCV, VGCV is a substrate for peptide transporters in the intestinal wall, explaining its enhanced bioavailability when given orally [41]. In adult studies oral bioavailability has been reported to be around 60% when taken with food (compared to 6%

for orally administered GCV). In adults GCV exposure, expressed as mean AUC_{24}, following 900 mg oral VGCV administration is comparable to that achieved with 5 mg/kg IV GCV [42].

Population pharmacokinetics in a phase II evaluation of VGCV solution conducted in congenitally infected neonates suggested a dose of 16 mg/kg twice daily as giving equivalent drug exposure, measured using AUC, to the 6 mg/kg IV GCV used in earlier studies conducted by this same group. Very wide variation in AUC was noted [43]. One case report has commented on the need to adjust dosing of VGCV frequently to maintain adequate plasma levels [44].

The phase II study described above reported oral bioavailability of only 48% on day 6 which increased to 64% at day 36. It has been hypothesised that this is due to maturation of the GI and hepatic esterases involved with de-esterification of valGCV to active GCV with increasing post-conceptional age [37]. Due to the concurrent increase in renal clearance of GCV described above, overall AUC only decreased by 15% following a dose of oral VGCV, compared to the 41% decrease seen with administration of IV GCV. More recent data on the use of VGCV in a paediatric renal transplant population of 22 children identified creatinine clearance and bodyweight as individual factors influencing the apparent oral clearance [45].

2.1.3 CSF and CNS Penetration

The parameters affecting penetration of drug into the CSF and brain parenchyma are complex and are not well described for GCV [46]. Cerebrospinal fluid concentrations of GCV in adults indicate a penetration of 24–67% [47]. Data are very limited and there are no studies in the paediatric age group or neonates in whom differences in the blood–brain barrier or the transport systems involved may alter drug availability to this compartment. There are no existing data relating to penetration of GCV into CSF or brain parenchyma following oral VGCV administration. Data obtained for aciclovir and valaciclovir have shown stable levels in the CSF when compared to serum implying that the CSF may act as a reservoir for drug. This may imply that absolute peak serum levels of drug are not important and equivalent drug exposure measured by AUC may be sufficient [48]. These studies have also found that timing of sampling of CSF may also not be important once steady state has been achieved, as shown by similar CSF concentrations found after 6 days and after 6 months of oral treatment with valaciclovir. One of the key questions raised by the use of oral VGCV, particularly in unwell infants with CNS disease, is whether the same CSF levels are obtained using VGCV compared to IV GCV.

2.2 Pharmacodynamics

Two early studies found better virological responses with a higher dose of GCV, and/or increased therapy duration (one study compared 6 mg/kg twice daily dose of GCV to 4 mg/kg twice daily; the other compared 5 mg/kg twice daily for 2 weeks with 7.5 mg/kg twice daily for 2 weeks followed by 10 mg/kg three times weekly for 3 months): those babies achieving viral suppression in the urine at the end of the

treatment course in both these studies had a better neurological outcome [49, 50]. Other authors have reported on the increase in symptoms from CMV following termination of treatment associated with a corresponding increase in viral load [44].

In a recent study treating congenitally infected babies with VGCV only 6/18 viraemic babies were CMV PCR negative at the end of 6 weeks treatment [37]. In this study viral suppression correlated neither with clinical resolution nor with any of the pharmacokinetic parameters measured (C_{max}, AUC_{12}, C_{last}, T_{last}). However, C_{max} and AUC were correlated with decreased white cell count and absolute neutrophil count.

Cinque et al. reported that CMV DNA in CSF of seven adult HIV patients with CNS disease was undetectable by PCR at the end of 3 weeks GCV treatment in three patients with low-level virus detected at start of treatment and was decreased, but still detectable, in four patients with a higher starting value. At autopsy, virus was detectable in CNS by PCR in 4/4 patients, including three of those who had continued to have detectable CMV by PCR [51].

Preliminary data from our group have shown that during a standard course of antiviral treatment, viral load decreases but is rarely fully suppressed in blood, urine or saliva. Indeed in 15 babies treated with 6 weeks' GCV, virus was still detectable at the end of treatment in 8/12 (67%), 8/10 (80%) and 2/5 (40%) of those with blood, urine and saliva samples available (Luck, personal communication). Following treatment cessation a rebound in both urine and salivary viral load is observed, with an increase in viraemia; this has not been widely reported in the adult literature.

Viral half-life in the first week of treatment has been estimated to be around 2.37 days using data derived from a study by Kimberlin et al. ([37] and unpublished observations). This is similar to rates calculated in patients with HIV infection, or following liver transplant and bone marrow transplant (2.56, 2.36 and 1.52 days, respectively) [52]. With more frequent sampling, the half-life of decline in CMV in HIV-positive patients was actually closer to 0.9 days. This observation has also been replicated in preliminary results of our studies with the half-life of decline in CMV DNA in both urine and blood in the first 3 days of treatment being 0.56 and 1.81 days, respectively. The reason for the failure for virus to be fully suppressed in blood does not therefore seem explicable by a difference in viral half-life, certainly during this first phase of viral decline. Given the preliminary nature of these data, correlation with clinical outcome is not yet possible.

2.3 Resistance

Resistance in CMV is usually due to mutations in pUL97, which encodes the viral protein kinase, or in the viral polymerase, UL54, gene and is associated with low plasma levels of GCV [53–55]. The use of oral GCV, and the hypothesised subsequent exposure to low levels of drug for long periods, has been associated with increased emergence of resistance mutations and clinical treatment failure [53, 55]. Sub-optimal dosing of GCV in children may likewise select for viral resistance and subsequent treatment failure.

2.4 Drug Levels

Routine therapeutic drug monitoring of GCV is not performed as part of standard clinical care in adults as there has been no clear correlation demonstrated between drug levels and clinical efficacy. There remains a real paucity of data on drug levels following routine treatment in infants and children and the relationship between drug levels and clinical outcome. Pilot data from our group, in collaboration with the Bristol Antimicrobial Reference lab (BCARE), have shown that in 339 trough samples GCV levels are significantly lower in younger children and those aged 5–18 years when compared to adults (Fig. 1).

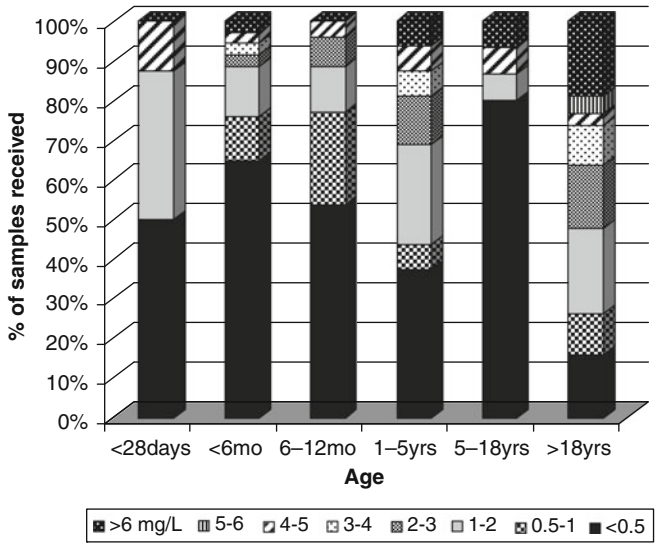

Fig. 1 Trough levels of ganciclovir (mg/L) in 192 children aged under 18 years compared to 147 adult GCV levels

As discussed above, there is a real concern that in older children sub-therapeutic levels may be common, leading to treatment failure and the development of resistance, particularly if longer treatment courses are being considered. In the congenitally infected group, monitoring treatment according to clinical and virological response poses a further challenge whereby the outcomes of interest are not immediately evident during treatment. The need for further data in this area is apparent.

2.5 Safety

Laboratory abnormalities reported during the Collaborative Antiviral Study Group (CASG) randomised study of IV GCV [56] were assessed utilizing NIAID Division of AIDS (DAIDS) toxicity tables. The most frequent adverse event was neutropenia,

which was experienced by a higher proportion of patients on the GCV arm of the study. Twenty-nine (63%) of 46 GCV-treated patients developed Grade 3 or 4 neutropenia during the 6 weeks of study drug administration, compared with 9 (21%) of 43 patients in the no-treatment group over the same period of time ($P < 0.01$). Dose adjustments were required for neutropenia in 14 (48%) subjects, although only four patients had the drug permanently discontinued. In all affected patients, the neutropenia resolved.

Anaemia was also more common in patients who received GCV (8 vs. 3% untreated patients), although the incidence of thrombocytopenia appeared comparable in the two treatment groups (2% GCV-treated patients vs. 3% untreated patients). The incidence of bloodstream infections, haematochezia and diarrhoea was also higher in patients who received GCV. Neurotoxicity, although very rare, has been documented in adults and is generally associated with poor renal function as GCV is almost completely renally excreted. Long-term side effects of treatment are also of potential concern in the paediatric age group. In animal models decreased spermatogenesis has been observed causing infertility. Increased tumours, lymphoma cell mutagenesis and DNA damage at doses lower than the anticipated human dose exposure have also been reported in animal models and in vitro (http://www.rocheusa.com/products/valcyte/pi.pdf; [57]).

In view of the concerns relating to carcinogenesis and gonadal toxicity, long-term follow-up of 47 children enrolled into a phase II trial of GCV as neonates between 1986 and 2001 has been conducted. Data relating to cancer incidence, sexual development and pubertal development in this small group of children is awaiting publication (http://clinicaltrials.gov/ct2/show/NCT00031421?term=ganciclovir+and+puberty&rank=1).

There are no other initiatives in place, to our knowledge, to conduct long-term monitoring of the subsequent development of neonates or older children treated with GCV or VGCV. We have recently developed a novel web-based pilot register for CMV-infected infants treated with GCV and VGCV. This is part of a pan-European treatment initiative to facilitate long-term post-treatment surveillance and linkage where possible of treated infants to cancer registries (www.ecci.ac.uk).

3 Clinical Studies of GCV in Childhood CMV Infection

3.1 Congenital CMV

Reports of treating congenitally infected neonates with severe CMV inclusion disease started to emerge in the mid-1990s. Case-based reports predominated and initially showed a trend towards improved outcome with treatment courses of IV GCV with publication bias being of possible concern (see Table 3).

Table 3 Summary of studies using ganciclovir to treat symptomatic congenital cytomegalovirus infection

Study	Patients	Dose GCV	Duration	Outcome	Side effects
Nigro [49]	12 infants	Gp 1 (*N*=6) 10 mg/kg/day	2 weeks	0/4 with persistent CMV DNA in urine had improved or normal outcome. Two babies in whom virus undetectable had clinical improvement and normal outcome	One patient in Gp 2 had decreased neutrophil count
		Gp 2 (*N*=6) 15 mg/kg/day IV then 10 mg/kg 3× per week	2 weeks then 3 months	All five children had clinical improvement	
Whitley et al. [50]	14 babies with symptomatic CMV involving CNS. <1-month old.	8 mg/kg/day IV	6 weeks	Mortality 4/47 (9%). 8/13 with retinitis resolved. No difference in neurological outcome. 12 mg/kg significantly improved viral suppression. Pharmacokinetics correlated with stabilised or improved hearing	9/47 (19% of children in each dose group) drug stopped due to toxicity. Dose modified in 6 (3 in each dose group). Neutropenia in 63% 8 mg/kg and 19% 12 mg/kg
	28 babies with CNS disease. <1 month	12 mg/kg/day IV			
Halwachs-Baumann et al. [16]	30 congenitally infected neonates: 21 treated (48% symptomatic). Nine no treatment (44% symptomatic)	10 mg/kg/day IV decreased to 5 mg/kg/day if side effects	Minimum 3 weeks	1/10 symptomatic children treated with antivirals symptomatic at 1 year compared to 2/3 symptomatic babies not treated. Large loss to follow-up	Three children drug dose decreased due to toxicity – not specified what

Table 3 (continued)

Study	Patients	Dose GCV	Duration	Outcome	Side effects
Michaels et al. [58]	9 children less than 1 year (5/9 started less than 10 days old).	10 mg/kg/day IV	2–4 weeks	No progression of hearing loss, 3/5 children improved hearing. Improvement in tone in three children	One child dose-dependent neutropenia, six children catheter-related problems
		5 mg/kg/day IV 550 mg/m^2/dose po GCV	5.8–18 months 8 months to 3 years		
Kimberlin et al. [56] (only randomised trial to date)	100 babies all with CNS disease. Enrolled less than 30 days old. 47 received drug, controls no treatment	12 mg/kg/day IV	6 weeks	At 1 year, 21% of treated babies worsening hearing compared to 68% untreated on best ear assessment	63% grade 3 or 4 neutropenia. 3 catheter infections
Rojo and Ramos [59]	Five babies less than 3 months of age all with neurological symptoms	8–12 mg/kg/day IV	6 weeks	No progression of symptoms	Neutropenia in child receiving 90 mg/kg/day
		30–90 mg/kg/day po GCV	6–12 months		
Tanaka-Kitajima [60]	Six cases of symptomatic congenital CMV (life-threatening or CNS involvement) D0–D45 (median D14)	5–12 mg/kg/day IV	2–7 weeks	All babies neurological deficits. Two babies hearing loss improved. Two patients with chorioretinitis improved, no visual loss at follow-up	Not reported

Reproduced from Expert Rev. Anti-infect. Ther. 4(6), 1005–1020 (2006) with permission of Expert Reviews Ltd. [36].

In 1997 Whitley et al. first reported an improved virological outcome with a dose of 6 mg/kg IV GCV twice daily when compared to 4 mg/kg twice daily, with a trend towards better neurological outcome in those receiving the higher dose [50]. The dose chosen in this initial study was based on pharmacokinetic data targeting a similar area under curve to that achieved in treatment regimes in adults [33]. Six weeks was chosen as the duration based purely on this being the considered upper limit of acceptability for intravenous therapy.

A randomised, although not placebo-controlled, study was subsequently conducted by the US CASG (CASG 106) in which 100 babies less than 1 month of age and of more than 32 weeks' gestation with evidence of CNS signs/symptoms of CMV disease were enrolled. These babies were randomised to receive either 6 weeks IV GCV or no treatment. Six weeks was chosen to be compatible with the previous randomised-controlled trial. In this study, which took 10 years to complete, none (0%) of 25 GCV recipients had worsening of hearing in their best ear between baseline and 6 months, compared with 7 (41%) of 17 patients in the no-treatment group [adjusted P-value < 0.001; OR 21.11 (95% CI: 2.84, ∞)]. Five (21%) of 24 GCV recipients had worsening of hearing in their best ear between baseline and $\geq$ 1 year, compared with 13 (68%) of 19 patients in the no-treatment group [adjusted P-value = 0.002; OR 10.26 (95% CI: 1.79, 81.92)]. GCV-treated patients also had a more rapid median time to normalisation of ALT (19 days) compared with patients in the no-treatment group (66 days) ($P = 0.03$) and a greater degree of weight gain ($P = 0.02$) and growth in head circumference ($P < 0.01$) at the end of 6 weeks treatment [56]. Importantly, subsequent analysis of neurological outcome has also shown, in the 74 infants with data available, that those who received antiviral treatment had fewer delays in the acquisition of expected developmental milestones (assessed using the Denver developmental assessment tool) compared to those in the untreated group [9.78 vs. 17.14 (P=0.007)] [61]. There are theoretical benefits in treating all symptomatic babies (not just those with CNS symptoms at birth), based on earlier cohort studies finding petechiae and IUGR to be independent risk factors for subsequent hearing loss [6]. In addition, the observation that some of the favourable outcomes seen in the earlier randomised-controlled study only reached significance in assessments close to the time of drug termination and the knowledge that neurological development continues for some years postnatally suggest that longer treatment courses are rational; such studies are now feasible with the availability of an oral preparation. A placebo-controlled, double-blind, randomised study (CASG 112) comparing 6 weeks vs. 6 months of oral VGCV has commenced recruitment in the USA (and will shortly start recruiting in Europe) aiming to address some of these issues.

Oral VGCV solution is now commercially available from Roche and licensed in Europe for treatment of CMV disease in patients aged over 18 years. The solution has good stability data and has a reasonable concentration of 50 mg/mL. This makes treatment outside formal studies possible and appealing to many clinicians. There is already increasing use of this drug in an off-label, unlicensed, manner in children in Europe including the prolonged treatment of babies infected with congenital CMV (personal communications). The use of oral VGCV allows earlier

discharge with treatment in the ambulatory setting, reducing costs and decreasing the complications associated with long-term venous access. The latter has special relevance for lower resource settings. However, recruitment into the CASG 112 study should be encouraged wherever possible (the USA and the UK) to provide the evidence for or against using a longer duration of treatment than 6 weeks.

3.2 Clinical Use of GCV in Postnatal CMV Infection

The evidence for efficacy of treating premature infants with CMV is very sparse and is based purely on a few case reports [62, 63]. There are few data on treating postnatally infected, premature infants and pharmacokinetic data only exist for those treated for congenitally acquired infection aged more than 32/40. A number of authors have described benefit in using GCV for treatment of neonatal hepatitis and cholestasis and gastrointestinal manifestations [64, 65]. The studies already discussed above found a more rapid normalisation of AST in congenitally infected babies, although all subjects (including those not receiving treatment) had eventual resolution of liver enzyme abnormalities [56]. Other authors have observed that babies either controlled virus themselves, had spontaneous resolution of their symptoms or that GCV treatment had no obvious benefit [23]. If treatment is commenced, GCV is the standard of care. The optimal dose, duration and clinical efficacy are largely unknown. GCV remains the drug of choice on neonatal units, despite this almost complete lack of data.

3.3 CMV Disease and GCV Treatment in Older Immunocompromised Children

The strategy of pre-emptive therapy based on regular screening by CMV PCR and treatment of positive viraemia with GCV has been widely adopted and taken up by paediatric transplant units. Again there are very limited data at all on the efficacy of this approach and the optimal dose of GCV to use. Although a 12 mg/kg/day dose is used now for congenital CMV, in older children and adolescents the standard dose is 10 mg/kg/day.

Local transplantation treatment protocols vary, but in Europe include

- In HSCT
  a 2-week IV GCV period followed by oral VGCV (VGCV stopped when level of immunosuppression is decreased)
- In SOT
  prophylaxis in high-risk patients: oral VGCV (VGCV stopping when level of immunosuppression is decreased)
  pre-emptive treatment: a 2-week IV GCV period followed by oral VGCV based on detection of CMV PCR or antigenaemia.

There is a need for more detailed PK studies to provide improved dosing recommendations, monitoring and dose adjustment schedules in this age group.

4 Summary

Both GCV and VGCV are increasingly being used in babies and children. Despite this, there are major limitations in the evidence base underlying their clinical use. The recent failure of Maribavir in its pivotal phase III trial means that there is unlikely to be any new anti-viral treatment for CMV disease entering clinical practice for many years. The therapeutic market for CMV infection is small and GCV is now off patent. VGCV is only licensed in adults. Current agents have major toxicities. There is a need to develop long-term treatment registries to follow-up children treated in early childhood. These registers have major ethical, financial and regulatory difficulties. This review has highlighted the main areas of clinical disease and indications for treatment. The lack of key data in specific areas, especially premature infants, CSF penetration, surrogate markers of efficacy and data in older children has been outlined along with suggestions for future trials that need to be conducted to improve the evidence-based prescribing of antiviral therapy in children with CMV disease.

References

1. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol. 2007;17(5):355–63.
2. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007;17:253–76.
3. Griffiths PD, Baboonian C, Rutter D, Peckham C. Congenital and maternal cytomegalovirus infections in a London population. Br J Obstet Gynaecol. 1991;98:135–40.
4. Barbi M, Binda S, Caroppo S, Ambrosetti U, Corbetta C, Sergi P. A wider role for congenital cytomegalovirus infection in sensorineural hearing loss. Pediatr Infect Dis J. 2003;22:39–42.
5. Nance WE, Lim BG, Dodson KM. Importance of congenital cytomegalovirus infections as a cause for pre-lingual hearing loss. J Clin Virol. 2006;35:221–5.
6. Rivera LB, Boppana SB, Fowler KB, Britt WJ, Stagno S, Pass RF. Predictors of hearing loss in children with symptomatic congenital cytomegalovirus infection. Pediatrics. 2002;110: 762–7.
7. Noyola DE, Demmler GJ, Nelson CT, Griesser C, Williamson WD, Atkins JT, Rozelle J, Turcich M, Llorente AM, Sellers-Vinson S, Reynolds A, Bale JF Jr, Gerson P, Yow MD. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. J Pediatr. 2001;138:325–31.
8. Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet. 2000;355:2032–6.
9. Cope AV, Sweny P, Sabin C, Rees L, Griffiths PD, Emery VC. Quantity of cytomegalovirus viruria is a major risk factor for cytomegalovirus disease after renal transplantation. J Med Virol. 1997;52:200–5.
10. Stagno S, Reynolds DW, Tsiantos A, Fuccillo DA, Long W, Alford CA. Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cytomegalovirus infections. J Infect Dis 1975;132:568–77.

11. Bradford RD, Cloud G, Lakeman AD, Boppana S, Kimberlin DW, Jacobs R, Demmler G, Sanchez P, Britt W, Soong SJ, Whitley RJ. Detection of cytomegalovirus (CMV) DNA by polymerase chain reaction is associated with hearing loss in newborns with symptomatic congenital CMV infection involving the central nervous system. J Infect Dis. 2005;191:227–33.
12. Boppana SB, Fowler KB, Pass RF, Rivera LB, Bradford RD, Lakeman FD, Britt WJ. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. J Pediatr. 2005;146:817–23.
13. Lanari M, Lazzarotto T, Venturi V, Papa I, Gabrielli L, Guerra B, Landini MP, Faldella G. Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. Pediatrics. 2006;117:e76–83.
14. Walter S, Atkinson C, Sharland M, Rice P, Raglan E, Emery VC, Griffiths PD. Congenital cytomegalovirus: association between dried blood spot viral load and hearing loss. Arch Dis Child Fetal Neonatal Ed. 2008;93:F280–5.
15. Ross SA, Novak Z, Fowler KB, Arora N, Britt WJ, Boppana SB. Cytomegalovirus blood viral load and hearing loss in young children with congenital infection. Pediatr Infect Dis J. 2009;28:588–92.
16. Halwachs-Baumann G, Genser B, Pailer S, Engele H, Rosegger H, Schalk A, Kessler H, Truschnig-Wilders. M. Human cytomegalovirus load in various body fluids of congenitally infected newborns. J Clin Virol. 2002;25(Suppl 3):S81–S87.
17. Kohyama J, Kajiwara M, Shimohira M, Iwakawa Y, Okawa H. Human cytomegalovirus DNA in cerebrospinal fluid. Arch Dis Child. 1994;71:414–18.
18. Stagno S, Reynolds DW, Pass RF, Alford CA. Breast milk and the risk of cytomegalovirus infection. N Engl J Med. 1980;302:1073–6.
19. Vochem M, Hamprecht K, Jahn G, Speer CP. Transmission of cytomegalovirus to preterm infants through breast milk. Pediatr Infect Dis J. 1998;17:53–8.
20. Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet. 2001;357:513–18.
21. Hamprecht K, Maschmann J, Jahn G, Poets CF, Goelz R. Cytomegalovirus transmission to preterm infants during lactation. J Clin Virol. 2008;41:198–205.
22. Kumar ML, Nankervis GA, Cooper AR, Gold E. Postnatally acquired cytomegalovirus infections in infants of CMV-excreting mothers. J Pediatr. 1984;104:669–73.
23. Cheong JL, Cowan FM, Modi N. Gastrointestinal manifestations of postnatal cytomegalovirus infection in infants admitted to a neonatal intensive care unit over a five year period. Arch Dis Child Fetal Neonatal Ed. 2004;89:F367–9.
24. Paryani SG, Yeager AS, Hosford-Dunn H, Johnson SJ, Malachowski N, Ariagno RL, Stevenson DK. Sequelae of acquired cytomegalovirus infection in premature and sick term infants. J Pediatr. 1985;107:451–6.
25. Vollmer B, Seibold-Weiger K, Schmitz-Salue C, Hamprecht K, Goelz R, Krageloh-Mann I, Speer CP. Postnatally acquired cytomegalovirus infection via breast milk: effects on hearing and development in preterm infants. Pediatr Infect Dis J. 2004;23:322–7.
26. Emery VC, Hassan-Walker AF, Burroughs AK, Griffiths PD. Human cytomegalovirus (HCMV) replication dynamics in HCMV-naive and -experienced immunocompromised hosts. J Infect Dis 2002;185:1723–8.
27. Cope AV, Sabin C, Burroughs A, Rolles K, Griffiths PD, Emery VC. Interrelationships among quantity of human cytomegalovirus (HCMV) DNA in blood, donor-recipient serostatus, and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation. J Infect Dis 1997;176:1484–90.
28. Gor D, Sabin C, Prentice HG, Vyas N, Man S, Griffiths PD, Emery VC. Longitudinal fluctuations in cytomegalovirus load in bone marrow transplant patients: relationship between peak virus load, donor/recipient serostatus, acute GVHD and CMV disease. Bone Marrow Transplant. 1998;21:597–605.

29. Jeena PM, Coovadia HM, Chrystal V. Pneumocystis carinii and cytomegalovirus infections in severely ill, HIV-infected African infants. Ann Trop Paediatr. 1996;16:361–8.
30. Rabie H, de Boer A, van den Bos S, Cotton MF, Kling S, Goussard P. Children with human immunodeficiency virus infection admitted to a paediatric intensive care unit in South Africa. J Trop Pediatr. 2007;53:270–3.
31. McNally LM, Jeena PM, Gajee K, Thula SA, Sturm AW, Cassol S, Tomkins AM, Coovadia HM, Goldblatt D. Effect of age, polymicrobial disease, and maternal HIV status on treatment response and cause of severe pneumonia in South African children: a prospective descriptive study. Lancet. 2007;369:1440–51.
32. O'Connell N, Rabie H, Freeman N, Cotton M Cytomegalovirus retinitis in HIV-1 infected South African children. 2009. In 5th International AIDS Society Conference on pathogenesis and treatment, Cape Town, South Africa.
33. Trang JM, Kidd L, Gruber W, Storch G, Demmler G, Jacobs R, Dankner W, Starr S, Pass R, Stagno S et al. Linear single-dose pharmacokinetics of ganciclovir in newborns with congenital cytomegalovirus infections. NIAID Collaborative Antiviral Study Group. Clin Pharmacol Ther. 1993;53:15–21.
34. Zhou XJ, Gruber W, Demmler G, Jacobs R, Reuman P, Adler S, Shelton M, Pass R, Britt B, Trang JM, Whitley RJ, Sommadossi JP. Population pharmacokinetics of ganciclovir in newborns with congenital cytomegalovirus infections. NIAID Collaborative Antiviral Study Group. Antimicrob Agents Chemother. 1996;40:2202–5.
35. Pacifici GM. Pharmacokinetics of antivirals in neonate. Early Hum Dev. 2005 Sep;81(9): 773–80.
36. Luck S, Sharland M, Griffiths P, Jenkins S. Advances in the antiviral therapy of herpes virus infection in children. Amend Expert Rev Anti Infect Ther. 2006;4(6):1005–20.
37. Kimberlin DW, Acosta EP, Sanchez PJ, Sood S, Agrawal V, Homans J, Jacobs RF, Lang D, Romero JR, Griffin J, Cloud GA, Lakeman FD, Whitley RJ. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease. J Infect Dis 2008;197:836–45.
38. Jacqz-Aigrain E, Macher MA, Sauvageon-Marthe H, Brun P, Loirat C. Pharmacokinetics of ganciclovir in renal transplant children. Pediatr Nephrol. 1992;6:194–6.
39. Zhang D, Lapeyraque AL, Popon M, Loirat C, Jacqz-Aigrain E. Pharmacokinetics of ganciclovir in pediatric renal transplant recipients. Pediatr Nephrol. 2003;18:943–8.
40. Vethamuthu J, Feber J, Chretien A, Lampe D, Filler G. Unexpectedly high inter- and intrapatient variability of ganciclovir levels in children. Pediatr Transplant. 2007;11: 301–5.
41. Sugawara M, Huang W, Fei YJ, Leibach FH, Ganapathy V, Ganapathy ME. Transport of valganciclovir, a ganciclovir prodrug, via peptide transporters PEPT1 and PEPT2. J Pharm Sci. 2000;89:781–9.
42. Razonable RR, Paya CV. Valganciclovir for the prevention and treatment of cytomegalovirus disease in immunocompromised hosts. Expert Rev Anti Infect Ther. 2004;2:27–41.
43. Acosta EP, Brundage RC, King JR, Sanchez PJ, Sood S, Agrawal V, Homans J, Jacobs RF, Lang D, Romero JR, Griffin J, Cloud G, Whitley R, Kimberlin DW. Ganciclovir population pharmacokinetics in neonates following intravenous administration of ganciclovir and oral administration of a liquid valganciclovir formulation. Clin Pharmacol Ther. 2007;81: 867–72.
44. Meine Jansen CF, Toet MC, Rademaker CM, Ververs TF, Gerards LJ, van Loon AM. Treatment of symptomatic congenital cytomegalovirus infection with valganciclovir. J Perinat Med. 2005;33:364–6.
45. Zhao W, Baudouin V, Zhang D, Deschenes G, Guellec CL, Jacqz-Aigrain. E. Population pharmacokinetics of ganciclovir following administration of valganciclovir in paediatric renal transplant patients. Clin Pharmacokinet. 2009;48:321–8.
46. Strazielle N, Ghersi-Egea JF. Factors affecting delivery of antiviral drugs to the brain. Rev Med Virol. 2005;15:105–33.

47. Fletcher C, Sawchuk R, Chinnock B, de Miranda P, Balfour HH Jr. Human pharmacokinetics of the antiviral drug DHPG. Clin Pharmacol Ther. 1986;40:281–6.
48. Lycke J, Malmestrom C, Stahle L. Acyclovir levels in serum and cerebrospinal fluid after oral administration of valacyclovir. Antimicrob Agents Chemother. 2003;47:2438–41.
49. Nigro G, Scholz H, Bartmann U. Ganciclovir therapy for symptomatic congenital cytomegalovirus infection in infants: a two-regimen experience. J Pediatr 1994;124(2): 318–22.
50. Whitley RJ, Cloud G, Gruber W, Storch GA, Demmler GJ, Jacobs RF, Dankner W, Spector SA, Starr S, Pass RF, Stagno S, Britt WJ, Alford C Jr, Soong S, Zhou XJ, Sherrill L, FitzGerald JM, Sommadossi JP. Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a phase II study. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. J Infect Dis 1997;175:1080–6.
51. Cinque P, Baldanti F, Vago L, Terreni MR, Lillo F, Furione M, Castagna A, Monforte AD, Lazzarin A, Linde A. Ganciclovir therapy for cytomegalovirus (CMV) infection of the central nervous system in AIDS patients: monitoring by CMV DNA detection in cerebrospinal fluid. J Infect Dis 1995;171:1603–6.
52. Emery VC, Cope AV, Bowen EF, Gor D, Griffiths PD. The dynamics of human cytomegalovirus replication in vivo. J Exp Med. 1999;190:177–82.
53. Emery VC, Griffiths PD. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. Proc Natl Acad Sci USA. 2000;97:8039–44.
54. Boivin G, Goyette N, Rollag H, Jardine AG, Pescovitz MD, Asberg A, Ives J, Hartmann A, Humar A. Cytomegalovirus resistance in solid organ transplant recipients treated with intravenous ganciclovir or oral valganciclovir. Antivir Ther. 2009;14:697–704.
55. Limaye AP, Corey L, Koelle DM, Davis CL, Boeckh M. Emergence of ganciclovir-resistant cytomegalovirus disease among recipients of solid-organ transplants. Lancet 2000;356:645–9.
56. Kimberlin DW, Lin CY, Sanchez PJ, Demmler GJ, Dankner W, Shelton M, Jacobs RF, Vaudry W, Pass RF, Kiell JM, Soong SJ, Whitley RJ. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. J Pediatr. 2003;143:16–25.
57. Tomicic MT, Bey E, Wutzler P, Thust R, Kaina B. Comparative analysis of DNA breakage, chromosomal aberrations and apoptosis induced by the anti-herpes purine nucleoside analogues aciclovir, ganciclovir and penciclovir. Mutat Res. 2002;505:1–11.
58. Michaels MG, Greenberg DP, Sabo DL, Wald ER. Treatment of children with congenital cytomegalovirus infection with ganciclovir. Pediatr Infect Dis J 2003;22:504–8.
59. Rojo P and Ramos JT. Ganciclovir treatment of children with congenital cytomegalovirus infection. Pediatr Infect Dis J 2004;23:80–8.
60. Tanaka-Kitajima N, Sugaya N, Futatani T, Kanegane H, Suzuki C, Oshiro M, Hayakawa M, Futamura M, Morishima T and Kimura H. Ganciclovir therapy for congenital cytomegalovirus infection in six infants. Pediatr Infect Dis J 2005;24:782–5.
61. Oliver S, Cloud G, Sánchez P, Demmler G, Dankner W, Shelton M, Jacobs R, Vaudry W, Pass R, Soong S, Whitley R, Kimberlin D. Neurodevelopmental outcomes following ganciclovir therapy in symptomatic congenital cytomegalovirus infections involving the central nervous system. J Clin Virol. 2009 Dec;46(Suppl 4):S22–S26.
62. Muller A, Eis-Hubinger AM, Brandhorst G, Heep A, Bartmann P, Franz AR. Oral valganciclovir for symptomatic congenital cytomegalovirus infection in an extremely low birth weight infant. J Perinatol. 2008;28:74–6.
63. Takahashi R, Tagawa M, Sanjo M, Chiba H, Ito T, Yamada M, Nakae S, Suzuki A, Nishimura H, Naganuma M, Tominaga N, Moriuchi M, Moriuchi H. Severe postnatal cytomegalovirus infection in a very premature infant. Neonatology. 2007;92:236–9.
64. Fischler B, Casswall TH. Malmborg P and Nemeth A. Ganciclovir treatment in infants with cytomegalovirus infection and cholestasis. J Pediatr Gastroenterol Nutr. 2002;34:154–7.
65. Nigro G, Krzysztofiak A, Bartmann U, Clerico A, Properzi E, Valia S, Castello M. Ganciclovir therapy for cytomegalovirus-associated liver disease in immunocompetent or immunocompromised children. Arch Virol. 1997;142:573–80.