Cytomegalovirus after kidney transplantation in 2020 : moving towards personalized prevention

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ABSTRACT

Cytomegalovirus-related complications after kidney transplantation remain a substantial challenge. Rather than applying one preventive strategy to all at-risk patients, we can now adapt our strategy at the individual patient level. Antiviral prophylaxis or a strict preemptive strategy may be optimal for patients at the highest risk for CMV, while patients at lower risk may benefit particularly from preemptive monitoring and the administration of therapy only if needed. CMV-specific T-cell assays may be useful for further refining the pre-transplant determination of CMV risk, and for guiding decisions about antiviral therapy need or duration. An immunosuppressive regimen including an mTORi reduces CMV risk and may thus be an attractive option in some patients. New antiviral agents may further expand our therapeutic arsenal in the near future, and the prospects of CMV vaccination and adoptive T-cell therapy appear at the horizon.

INTRODUCTION

Cytomegalovirus infection remains an important challenge in the early phase after kidney transplantation. Without preventive measures, its complications range from asymptomatic viremia or mild flu-like symptoms to severe and sometimes life-treathening tissue-invasive disease, such as pneumonitis or colitis [1]. The term CMV infection is used to describe detectable CMV replication (e.g. DNAemia), whereas the term CMV disease is reserved for CMV infection with attributable symptoms [2]. CMV-seronegative recipients receiving a graft from a CMV-seropositive donor (D+/R−) are at highest risk.Data from the 1990s, before antiviral preventive strategies, reveal that in D+/R− transplants, about 70% of recipients developed CMV infection within 3 months after transplantation, and over 50% of recipients developed CMV disease [3]. On the other hand, CMV-seropositive transplant recipients (R+) have pre-existing immunity against CMV, but transplantation can lead to re-activation of the host's virus. Additionally, when a CMV-seropositive recipient receives a graft from a CMV-seropositive donor (D+/R+), there is a risk of superinfection with a different CMV strain. Without preventive measures, CMV-seropositive recipients are generally considered to have a moderate 10–20% risk of developing CMV disease [9, 10], with this risk being somewhat higher in D+/R+ than D−/R+ transplantations [5].

Importantly, the type and intensity of immunosuppressive therapy also influences the risk of CMV-related complications. Compared to no induction, induction therapy with antithymocyte globulin (ATG) increases the risk of CMV infection by 50% [6]. In contrast, monoclonal interleukin 2 receptor antibodies do not seem to increase CMV infection risk [7]. Maintenance regimens including mammalian target of rapamycin inhibitors (mTORi) are reported to markedly reduce CMV risk [8–10].

In the era before antiviral preventive strategies became available, post-transplantation CMV infection was associated with higher risks of opportunistic infections, acute rejection, graft loss, and death [11]. These so-called indirect effects of CMV are thought to be due to its immunomodulating effects, and the chronic low-grade viral persistence in the allograft [12]. Despite routine use of

preventive strategies nowadays, several recent studies still confirm the negative impact of CMV on kidney transplant outcomes such as acute rejection, graft survival, mortality and cardiovascular events[13–16]. An analysis of national US data—including over 50,000 deceased donor kidney transplants performed between 2010 and 2015—revealed that compared to D−/R− transplantations, D+/R− transplantations were associated with increased risks of graft loss [hazard ratio (HR) = 1.17, P = 0.01], all-cause mortality (HR = 1.18, P < 0.001), and infection-related mortality (HR = 1.38, P = 0.03), although the early graft rejection rates were similar [17]. However, this registry analysis lacked data regarding CMV prevention strategies and development of CMV infection or disease, thus preventing analysis of the direct association between CMV serostatus, CMV infection, and transplant outcomes. On the other hand, a very recent report provides long-term data from a well-defined Swiss single-centre cohort (n = 599) managed using a standardized CMV prevention/treatment protocol, which shows no difference in rejection risk, graft, or patient survival according to CMV serostatus [4]. They also used a multivariable Cox proportional hazards model, and found that CMV serostatus, CMV replication, and CMV disease were not independent predictors of patient death or graft failure [4]. These apparently contradictory findings may be related to the high heterogeneity amongst populations—including diverse immunosuppressive drug regimens, CMV prevention strategies, and CMV treatment options.

PREVENTIVE ANTIVIRAL DRUG STRATEGIES: PROPHYLAXIS OR PREEMPTIVE?

The development of antiviral drugs—starting with aciclovir (ACV) and ganciclovir (GCV) in the 1980s, and followed by the introduction of valganciclovir (VGC) and valaciclovir (VAC) in the early 2000s—enabled the development of strategies to prevent CMV after kidney transplantation. There are currently two commonly used strategies: universal prophylaxis and preemptive therapy [18]. Universal prophylaxis involves administering antiviral therapy to all at-risk patients (e.g. all recipients in D+/R− and R+ transplantations, or a specific subset), starting during the early post-transplant

period and continuing for a defined duration (e.g. 3–6 months). In preemptive therapy, patients are monitored at regular intervals (often weekly) for early evidence of CMV replication in blood using a laboratory assay, most often a polymerase chain reaction (PCR) test. Once a predefined threshold is reached, antiviral drug treatment is initiated to prevent evolution to symptomatic disease, and continued until CMV DNAemia disappears. Several randomized controlled trials have directly compared outcomes between prophylaxis versus preemptive therapy. Meta-analysis of pooled data from these trials revealed that preemptive and prophylactic therapy did not yield significant differences in CMV disease, mortality, graft loss, acute rejection, or infections; however, prophylaxis was associated with significantly more leucopenia [19]. Largely based on these findings, the current consensus guidelines from the Transplantation Society International CMV Consensus Group recommend the use of either prophylaxis or preemptive therapy for recipients in both D+/R− and R+ transplantations, except when very strong immunosuppression is used, e.g. antithymocyte globulin (ATG), in which case they suggest to prefer prophylaxis [20]. Nevertheless, it is notable that the trials underpinning these recommendations were mostly small, and were highly heterogeneous. Table 1 presents an overview of all RCTs comparing prophylaxis versus preemptive therapy in kidney transplant recipients.

Insert Table 1

The studies listed in Table 1 used different drugs, drug dosages, and treatment durations. The preemptive protocols differed in terms of CMV detection methods, cut-offs for starting treatment, monitoring intervals, and treatment durations. Moreover, different immunosuppressive therapies were used (e.g. some included ATG induction). Importantly, the largest study only included R+ transplantations [5], and thus there are particularly limited data regarding the high-risk D+R− transplantations: in 5 of 6 evaluable studies, D+/R− transplantations constitute 93 (14%) of 672 cases.

Notably, the conclusions of these studies are somewhat diverging, with some showing superiority of prophylaxis and others reporting equivalent results in terms of preventing CMV disease. However, it should be noted that the trials by Kliem et al. [25] and Witzke et al. [5], which

suggested superiority of prophylaxis, used a suboptimal monitoring strategy in the preemptively managed group with long (≥ 2 weeks) monitoring intervals from the second month on, whereas the trials by Khoury et al. [23] and Reischig et al. [24] which used more intense preemptive monitoring showed equivalent results. Of note, a recent RCT -although in liver transplants- even showed superior results with preemptive therapy compared to prophylaxis [26]. Long-term follow-up results of the RCTs diverge as well, with Kliem's trial showing better graft survival with prophylaxis [25], whereas Reischig's trial indicated better graft survival with preemptive therapy [27], Khoury's trial noting a slighty higher mortality with prophylaxis [28] but Witzke's trial suggesting similar long-term outcomes [29]. Due to the major heterogeneity between the studies, it is impossible to draw solid conclusions regarding the relative effectiveness of prophylaxis or preemptive treatment to prevent CMV disease, especially in the D+/R− subgroup for which we have particularly limited data. This has resulted in an ongoing debate about the preferred strategy, which is reflected by the varying approaches between centres. A recent survey showed that almost all centres worldwide (93%) use some form of preventive strategy for CMV, most commonly with VGC as the antiviral agent: 46% use universal prophylaxis, 21% use preemptive therapy, and 33% use a split approach depending on the recipient's CMV risk [18].

Preemptive therapy can be successfully used, even in D+/R- patients [23, 24, 26, 30] but requires stringent logistic conditions. Monitoring should be performed weekly during the first 3 -4 months [20] and the threshold value for treatment should be low, especially in the D+/R- subgroup where viral load replication evolves more rapidly [30, 31]. Prompt initiation of preemptive treatment is crucial and therefore requires excellent compliance by both the patient and the medical team. Repeated treatment is sometimes needed, especially in high-risk patients [30, 32]. Prophylaxis, at first sight, looks like an easier alternative. However, prophylaxis also has drawbacks. First, there is the risk of late-onset disease. Although prophylaxis efficiently controls CMV replication during treatment, it may hamper the development of a CMV-specific immune response, potentially resulting in CMV disease when prophylaxis is stopped [26]. The IMPACT trial—a randomized controlled trial comparing two

durations of VGC prophylaxis in D+/R− kidney transplant recipients—found late-onset CMV disease in 37% of the patients with 100 days of prophylaxis, and in only 16% of patients with 200 days of prophylaxis [33]. Late-onset disease can be problematic because its diagnosis is often delayed due to the non-specificity of the symptoms and less frequent medical visits beyond 3 months post-transplantation. Although most late-onset disease manifestations are limited to mild-to-moderate flu-like symptoms, some cases have exhibited severe disease, and increased risk of graft loss and mortality have been described [34]. A second major drawback of VGC prophylaxis is the high risk of leucopenia. The IMPACT trial reported a 26–38% risk of leucopenia, depending on prophylaxis duration, including grade 3–4 leucopenia in 14% of patients [33]. Forteen % of patients in each arm of the trial developed neutropenia. Overall, 13–14% of patients required treatment with granulocyte-colony stimulating factor (GCSF). Other centres have reported up to 35% incidence of neutropenia during VGC prophylaxis [35]. Importantly, neutropenia may increase the risks of other complications, such as infections and acute rejection [36]. The large variation in neutropenia incidence during VGC prophylaxis may be explained by differences in VGC dosage, and in the concomitant use of drugs with haematotoxic side-effects, e.g. ATG or mycophenolic acid (MPA). Some centres use half the recommended dose to reduce costs and side-effects. Several retrospective observational studies have compared outcomes between normal-dose and low-dose VGC prophylaxis [37–39]. In R+ transplantations, the data suggest that low-dose and normal-dose VGC have similar efficacy. The findings are less clear in D+/R− patients. In this subgroup, there is particular concern that low-dose VGC might lead to breakthrough disease and resistance development [37]. Therefore, current consensus guidelines do not recommend routine use of low-dose VGC [20]. A final consideration regarding VGC prophylaxis relates to its possible association with polyomavirus nephropathy (PVAN). A recent post-hoc analysis on 2 RCTs evaluating different CMV prevention regimens [24, 35] showed that the incidence of both BK viremia and PVAN was increased in patients treated with VGC prophylaxis compared to those receiving VAC prophylaxis or preemptive therapy [40]. Given that the authors also found less rejection and late graft fibrosis with VGC prophylaxis compared to VAC [35, 41]

and further supported by several in-vitro studies [42–45], the authors speculate that VGC may have immunosuppressive properties. More data are needed to confirm this intriguing hypothesis.

The current consensus guidelines do not specifically mention a split approach, in which high-risk patients receive prophylaxis, while moderate-risk patients are preemptively managed [20]. However, this option may be attractive because it restricts long-term exposure to antiviral drugs to those who are most likely to benefit (D+/R− patients), and limits drug toxicity and costs for patients at lower risk of developing CMV complications (R+ patients). Thus, the split approach represents a first step towards personalized treatment.

ROLE OF mTORi IN CMV PREVENTION?

Data consistently show that regimens including mTORi reduce the post-transplantation CMV risk. A recent systematic review and meta-analysis of RCTs reported that CMV infection rates were lower with mTORi-based regiments than calcineurin-inhibitor (CNI)-based regimens [risk ratio (RR), 0.54; 95% confidence interval (CI), 0.41 to 0.72], and lower in regimens containing mTORi plus a reduced dose of CNI than in regimens including a regular dose of CNI (RR, 0.43; 95% CI, 0.24 to 0.80) [8]. After publication of this systematic review in 2017, two others large RCTs confirmed that CMV infections were reduced by regimens using mTORi plus CNI. In the TRANSFORM trial (n = 2037), the CMV infection risk was over 2-fold lower in the everolimus (EVL) plus reduced-exposure CNI arm compared to the arm receiving MPA and standard-exposure CNI (8.1% versus 20.1%, P < 0.001), and this difference was increased to over 3-fold among high-risk (D+/R−) patients [15, 36]. In the ATHENA trial (n = 655), a similar reduction of CMV infections was observed in patients randomized to EVL plus standard-exposure CNI compared to patients receiving MPA plus standard-exposure CNI [10]. From these clinical trials, it is difficult to discern whether mTORi have an intrinsic antiviral effect, or if the mTORi-containing immunosuppressive regimen delivered a somewhat weaker overall immunosuppressive potency than its comparators. Nevertheless, substantial experimental evidence

supports that mTORi have antiviral properties, which are ascribed to a variety of direct and indirect mechanisms [47].

The reported efficacy of regimens containing mTORi plus CNI in terms of graft function and rejection have increased the popularity of such therapy. It is a particularly attractive option for patients with high CMV risk (D+/R−), not only because it strongly reduces the risk of CMV-related complications, but also because it decreases the neutropenia risk compared to regimens using MPA [9]. This could promote better tolerance of VGC treatment. Some even argue that VGC prophylaxis may be dispensable when using an mTORi-based regime, due to the reduced risk of CMV, such that preemptive monitoring could be applied even for D+/R− patients [48]. However, the presently available data are insufficient to estimate the CMV risk in D+/R− patients receiving mTORi without CMV prophylaxis, and additional studies are needed before this strategy can be advised.

Switching to mTORi treatment during post-transplant follow-up may also be an attractive option when a patient develops CMV-related complications, but this also requires more research. In a currently ongoing prospective randomized trial, kidney transplant recipients are randomized after a first CMV episode to either conversion to a regimen with sirolimus and low-dose Tac, or to maintaining a regimen with azathioprine or MPA plus Tac (NCT02671318 on clinicaltrials.gov).

CMV-SPECIFIC CELL-MEDIATED IMMUNITY ASSAYS: WHERE DO WE STAND?

Currently, prevention strategies are universally applied either to all at-risk patients or to specific subgroups. However, some of these patients would never develop CMV complications even without any prevention, and thus there is overconsumption of diagnostics and unnecessary antiviral drug exposure in some cases. On the other hand, some patients still develop CMV-derived complications despite preventive measures. In recent years, to improve predictions of the risk for developing CMV infection or disease, multiple diagnostic assays have been developed to measure CMV-specific T-cell immunity. Indeed, the adaptive immune response, predominantly of T lymphocytes, is critical for

controlling CMV replication [49], but this response is severely hampered in the early post-transplant period. Many factors influence the time it takes to (re)establish CMV immunity, including the type and strength of immunosuppressive therapy and the use of antiviral prophylaxis [50].

There are a variety of CMV-specific cell-mediated immunity (CMI) assays, some of which are already commercially available in the clinical setting. The QuantiFERON-CMV® assay is an enzyme-linked immunosorbent-based assay (ELISA) that measures interferon gamma (IFN-γ) release, mostly by CMV-specific CD8+ T cells, after in vitro stimulation of whole blood using a pool of 22 immunogenic viral peptides. The T-Track CMV® and T-SPOT.CMV® assays are enzyme-linked immunosorbent spot (ELISPOT) tests that detect the IFN-γ-producing response in individual cells upon stimulation with specific CMV peptides. Other assays are currently reserved for research purposes, such as intracellular cytokine staining for IFN-γ using flow cytometry, and major histocompatibility complex multimer staining [22, 39].

Overall, the available CMI assays reveal that the quantity and quality of virus-specific T cells are inversely correlated with viral replication, and strong cellular immune responses are associated with containment of viral replication [50]. Thus, a negative test may indicate insufficient T-cell immunity against CMV and hence a higher risk of CMV complications, whereas a positive test may indicate protection. Several observational studies have consistently shown that these tests predict CMV DNAemia and disease, and might be useful in various clinical scenarios, such as for pretransplant risk stratification of R+ patients, guidance regarding preemptive therapy initiation or duration, or identifying those at risk for late-onset CMV disease after stopping prophylaxis [51].

The key obstacles to introducing these T-cell immunoassays into clinical practice have been the lack of reliable cut-offs for clinical decision-making, and the paucity of interventional trials justifying changes of antiviral treatment or immunosuppression. Very recently, a large multicenter trial derived a threshold value for the T-SPOT.CMV® assay to indicate protection from CMV events after completion of prophylaxis [52]. This established threshold was mainly driven by the R+ group. In

the D+/R− subgroup, however, most patients had no detectable CMV-specific CMI by the end of antiviral prophylaxis, and a negative test had a poor ability to predict subsequent CMV events, therefore limiting its usefulness in this particular setting.

Interventional studies, where treatment decisions are made in real-time based on the results of a given CMI assay, are needed to demonstrate if the CMI assay can improve patient management in a feasible, safe and cost-effective way. A first proof-of-concept study using the QuantiFERON-CMV® assay suggests that immune monitoring could be useful for deciding when to stop prophylaxis, although these findings are very preliminary and require further evaluation in much larger studies with strict protocols [53]. Several other interventional studies are currently ongoing.

NEW ANTI-CMV DRUGS IN THE PIPELINE

Although valganciclovir is a very potent anti-CMV drug, its side-effects, especially leucopenia and neutropenia, remain an important obstacle to CMV prevention and treatment. Moreover, drug resistance sometimes develops [54]. Other compounds are under investigation, in hopes of finding a less toxic alternative to VGC for CMV prevention, or a rescue therapy for CMV disease in cases of ganciclovir resistance. Letermovir demonstrated efficacy and safety as a CMV prophylactic agent in hematopoietic stem cell transplant recipients (HSCT) compared to placebo, and was generally well-tolerated, with no signs suggesting myelotoxicity or nephrotoxicity [55]. A large international phase III RCT is currently ongoing, comparing prophylaxis with letermovir versus VGC in D+/R− kidney transplant recipients (NCT03443869). A second promising compound is maribavir. In early studies, low-dose maribavir failed to demonstrate efficacy for prophylaxis in D+/R− liver transplant recipients [56]. However, a recent phase II RCT used higher doses of maribavir as preemptive therapy in recipients of hematopoietic stem cell and solid-organ transplants, revealing efficacy similar to that of VGC in clearing CMV-DNAemia [57]. Maribavir was associated with less neutropenia, but more gastrointestinal adverse events, particularly dysgeusia. Phase III RCTs are currently ongoing to assess

maribavir's efficacy for treatment of refractory/resistant CMV infection in hematopoietic stem cell and solid-organ transplant recipients (NCT02927067, NCT02931539). Finally, brincidofovir, a prodrug of cidofovir with broad antiviral activity, has exhibited preliminary evidence of efficacy in a placebo-controlled phase III trial of CMV prophylaxis in HSCT recipients [58]; however, significant toxicity especially diarrhoea, led to the halt of planned trials in kidney transplant patients. An intravenous formula is being developed with the aim of eliminating the gastro-intestinal side-effects.

THE ELUSIVE SEARCH FOR A CMV VACCINE

Since the 1970s, active research has been conducted to develop a CMV vaccine, but this has proved to be very challenging because the virus is a master at immune evasion. Despite the investigation of numerous potential vaccines in pre-clinical and early clinical studies, none is in active phase III [59]. In a recent phase II trial, the DNA-based vaccine ASP0113 was administered to D+/R− kidney transplant recipients during the early post-transplant period, but unfortunately showed no efficacy [60]. A trial of this vaccine in HSCT recipients also yielded negative results (NCT01877655). Most recently, a poxvirus-vectored vaccine showed preliminary evidence of efficacy in a phase II trial in HSCT recipients. Additional studies with this vaccine in the HSCT setting are ongoing (NCT03560752, NCT03354728), and plans exist to expand the research to solid organ transplants. Several other vaccines are currently entering phase II (NCT03486834, NCT03629080, NCT02396134).

ADOPTIVE CMV-SPECIFIC T-CELL THERAPY

Adoptive CMV-specific T-cell therapy is a potential alternative to classic antiviral therapy for patients with refractory or resistant CMV infection. It has been successfully used in allogeneic HSCT recipients, with CMV-specific T cells classically derived from the donor [61]. However, sparse data are available from solid organ transplant (SOT) recipients. Obviously, it is more difficult to manufacture effective

CMV-specific T cells using autologous immune cells from heavily immunosuppressed patients. Nevertheless, a recent first phase I trial used in vitro-expanded autologous CMV-specific cells in SOT recipients with recurrent or ganciclovir-resistant infection, and reported encouraging results [62]. Of 13 treated patients, 11 (84%) exhibited showed improvement in symptoms, including complete resolution or reduction in DNAemia and CMV-associated end-organ disease and/or cessation or reduced use of antiviral drugs. Furthermore, this adoptive immunotherapy carried no serious adverse events. However, one drawback is that it takes several weeks to produce these patient-derived effector cells. One attractive alternative is the use of readily available HLA-matched third-party banked cells, which also has the potential to be commercialized. Although treatment with "off-the-shelf" third-party virus-specific T cells has shown promising results in HSCT recipients [63], the safety and efficacy in SOT recipients remains unclear.

CONCLUSIONS

Cytomegalovirus-related complications remain an important challenge after transplantation, which is reflected by the intense ongoing research in this field. Rather than applying one preventive strategy to all at-risk patients, we can now potentially adapt our strategy at the individual patient level. At the time of transplantation, a first estimate of CMV risk can be made based on D/R serostatus and the need for T-cell-depleting induction therapy. This risk estimation may be further refined by performing pretransplant or early post-transplant CMV-specific T-cell assays. In patients estimated to be at high CMV risk, both VGC prophylaxis or a strict preemptive strategy are valid options, and one could consider an immunosuppressive regimen comprising mTORi. CMV-specific T-cell monitoring may help guide the duration of prophylaxis or determine which patients with low-level DNAemia require treatment. In patients at lower CMV risk, long-term exposure to potentially toxic antiviral prophylaxis could be avoided. In this setting, patients may benefit from close follow-up through preemptive monitoring, and therapy only if needed. New antiviral agents may further expand our therapeutic arsenal in the near future, and the prospects of CMV vaccination and adoptive T cell therapy appear at the horizon.

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Table 1. Overview of randomized controlled trials comparing a pre-emptive strategy versus antiviral prophylaxis for prevention of CMV disease after kidney transplantation

	Country	N	D+/R−	R+	T-cell depleting induction	FU	Pre-emptive (PE) regimen	Prophylaxis	Detection method	Symptomatic CMV disease PE vs prophylaxis	Leucopenia PE vs prophylaxis
Jung, 2001 [21]	Germany	70	10	Not specified	0%	12 mo	Oral GCV 3000 mg/d for 14 days or until test negative. Monitoring: ≥2×/week in month 1, 1×/week in months 2–3, 1×/2 weeks in months 4–5, monthly thereafter. Threshold for therapy: 2/200,000 pp65-positive leukocytes and/or PCR 400 CMV copies/mL.	Oral GCV 3000 mg/d for 90 days	Pp65 antigenemia or PCR	8% vs 9% (NS)	50% prematurely stopped prophylaxis due to side effects (9/34 had leucopenia or thrombopenia)
Queiroga, 2003 [22]	Brazil	34	Not specified	Not specified	100%	6 mo	Oral GCV (dose/route not specified).	GCV 750 mg 3×/day for 90 days	Pp65 antigenemia	0% vs 0%	NA
Khoury, 2006 [23]	USA	98	29	69	97%	12 mo	Oral VGC 900 mg 2×/day for at least 21 days or until test negative. Weekly monitoring until week 16. Threshold for therapy: whole blood qPCR > 2000 copies/mL.	Oral VGC 900 mg/day for 100 days	Whole blood qPCR	1% vs 8% (P = 0.4)	Neutropenia: 2% vs 4%
Reischig, 2008 [24]	Czech Republic	70	10	60	13%	12 mo	Oral VGC 900 mg 2×/day for at least 14 days or until test negative. Weekly monitoring until week 16. Threshold for therapy: whole blood qPCR > 2000 copies/mL.	Oral VAC 2 g 4×/day for 3 months	Whole blood qPCR	6% vs 9% (P = 0.6)	Leucopenia: 17% vs 32% Neutropenia: 14% vs 29% GCSF: 6% vs 18%
Kliem, 2008 [25]	Germany	138	44	94	11%	48 mo	IV GCV 5 mg/kg 2×/day for at least 10 days or until test was <100 copies CMV DNA/mL on two successive tests. Monitoring: weekly in weeks 1–4, every two weeks in weeks 5–12, monthly in weeks 13–52, every 3 months thereafter. Threshold for therapy ≥400 copies/mL.	Oral GCV 1000 mg/d for 90 days	Whole blood qPCR	19% vs 7% (P < 0.05)	Leucopenia 1% vs 15% Neutropenia 0% vs 1%
Witzke, 2012 [5]	Germany/ Austria	296	0	296	4%	12 mo	Oral VGC 900 mg 2×/day for at least 14 days or until test negative (< 400 copies/mL) with secondary prophylaxis of VGC 450 mg 2×/d for 28 days. Monitoring: weekly in weeks 1–4, every 3 weeks in weeks 6–28, every 3 months thereafter. Threshold for therapy ≥400 copies/mL.	Oral VGC 900 mg 2×/d for 100 days	Plasma qPCR	19% vs 4% (P = 0.003)	Leucopenia: 27% vs 36% Neutropenia: 5% vs 10%

FU, follow-up; mo, months