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Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Current Status, Known Challenges, and Future Strategies

Michael Boeckh,[1] W. Garrett Nichols,[1] Genovefa Papanicolaou,[2] Robert Rubin,[3] John R. Wingard,[4] John Zaia[5]

[1]Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, Washington; [2]Memorial Sloan-Kettering Cancer Center, New York, New York; [3]Harvard Medical School, Brigham and Women's Hospital and Harvard/Massachusetts Institute of Technology, Division of Health Sciences and Technology, Boston, Massachusetts; [4]University of Florida College of Medicine, Gainesville, Florida; [5]City of Hope National Medical Center and Beckman Research Institute, Duarte, California

Correspondence and reprint requests: Michael Boeckh, MD, Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, P.O. Box 19024, Seattle, WA 8109-1024 (e-mail: mboeckh@fhcrc.org).

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ABSTRACT

Cytomegalovirus (CMV) infection is a major cause of morbidity and mortality after hematopoietic stem cell transplantation. Significant progress has been made in the prevention of CMV disease over the past decade, but prevention of late CMV disease continues to be a challenge in selected high-risk populations. The pretransplantation CMV serostatus of the donor and/or recipient remains an important risk factor for posttransplantation outcome despite the use of antiviral prophylaxis and preemptive therapy; CMV-seropositive recipients of T cell–depleted grafts in particular continue to have a survival disadvantage compared with seronegative recipients with seronegative donors. The risk of developing antiviral drug resistance remains low in most patients; however, in a setting of intense immunosuppression (eg, after transplantation from a haploidentical donor), the incidence may be as high as 8%. Primary CMV infection via blood transfusion can be reduced by the provision of seronegative or leukocyte-depleted blood products; however, a small risk of 1% to 2% of CMV disease remains. Surveillance and preemptive therapy are effective in preventing the sequelae of transfusion-related CMV infection. Indirect immunomodulatory effects of CMV are increasingly recognized in hematopoietic stem cell transplant recipients. Strategies currently being investigated include long-term suppression of CMV with valganciclovir for the prevention of late CMV infection and disease, adoptive transfer of CMV-specific T cells, and donor and recipient vaccination strategies.

KEY WORDS

CMV • Herpes virus • Hematopoietic stem cell • Transplantation • Immunosuppression • Resistance

INTRODUCTION

Historically, untreated cytomegalovirus (CMV) infection and disease were associated with significant morbidity in the early period after transplantation and led to mortality in nearly 25% of seropositive patients undergoing allogeneic hematopoietic stem cell transplantation (SCT) [1-3]. Despite the introduction of effective antiviral therapies during the last 2 decades, the mortality from late CMV disease and the indirect effects of CMV remain deterrents to successful long-term outcomes [4,5]. Furthermore, as hematopoietic SCT has evolved, less toxic nonmyeloablative conditioning regimens coupled with intensive postengraftment immunosuppressive therapies have enabled the use of less selective transplant matches and have enabled older patients to undergo transplantation, with increasing risks for subsequent opportunistic and reactivated infections requiring further intervention.

Although significant progress has been made in diagnostic and prevention strategies, hematopoietic SCT recipients exist in a dynamic environment and

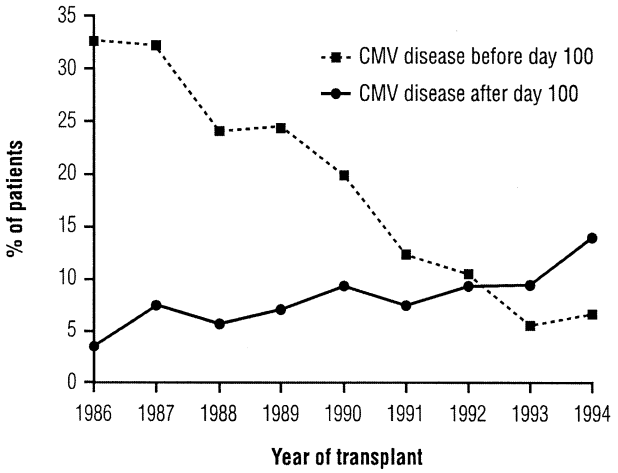

Figure 1. Incidence of early (before day 100) versus late (after day 100) cytomegalovirus (CMV) disease by year of hematopoietic stem cell transplantation in seropositive allogeneic recipients (n = 1458). Reprinted with permission [6].

require constant surveillance and reassessment of viral challenge. In particular, those high-risk recipients characterized by treatment with high-dose corticosteroids, mycophenolate mofetil, and T-cell depletion or anti–T-cell strategies are most vulnerable to CMV infection. Also, long-term outpatient management poses a more practical concern in some areas. During this review of our current knowledge of CMV, risk factors, prevention and treatment strategies, the potential threat of CMV drug resistance, and unresolved issues in our current prophylactic and therapeutic approaches will be discussed.

EFFECT OF CMV IN HEMATOPOIETIC SCT

Regardless of currently available antiviral strategies, hematopoietic SCT recipients remain at risk for CMV infection not only during the early posttransplantation period (<100 days), but also later (>100 days) in the posttransplantation course. Before the introduction of ganciclovir, the vast majority of CMV infections occurred in the time period between engraftment and day 100 after hematopoietic SCT, with sporadic occurrences before engraftment [6] (Figure 1). However, whereas the prevalence of early CMV disease has declined to 3% to 6% with intense antiviral drug use, the risk of late CMV disease has increased over the past few years, with up to 18% of recipients developing disease even when no prevention is administered [7-12].

Effect of CMV Serostatus in the Era of Preemptive Therapy and Prophylaxis

The issue of CMV serostatus in the ganciclovir era is complex and continues as an area of active study [13-21] (Table 1). Despite almost complete prevention of CMV disease in most of these studies, positive CMV serostatus of the recipient remains a poor prognostic factor, especially in recipients of T cell–depleted marrow or stem cells. An association of CMV with graft-versus-host disease (GVHD) and nonviral infections or sepsis has been suggested as a possible mechanism [13,18]. A large study by Ljungman et al. [16] demonstrated the importance of donor serostatus among CMV-seropositive recipients of unrelated grafts, possibly as a result of transferred CMV-specific immunity from the donor to the recipient. In an analysis of a large cohort of T cell–replete SCT recipients, both donor-positive/recipient-positive and donor-positive/recipient-negative recipients had a higher risk of mortality [18]. After controlling for neutropenia and CMV disease, only donor-positive/recipient-negative recipients had a higher risk of mortality. The authors attributed this outcome to indirect immunomodulatory effects of CMV because there was an excess mortality due to bacterial and fungal infections when compared with donor-negative/recipient-negative recipients [18]. Collectively, these studies of the effect of CMV serostatus in the ganciclovir era suggest that CMV infection before transplantation remains an important factor leading to poor outcome after transplantation, especially in recipients of T cell–depleted transplants.

Effect of Source of Stem Cells and CD34 Selection

Recipients of transplants from unrelated or mismatched related donors in some analyses show an increased risk of CMV disease, CMV-associated death, and transplant-associated mortality [23]. Whereas altering the cellular components of the hematopoietic SCT through CD34 depletion is theoretically advantageous in reducing contaminating tumor cells, T cell–depleting the graft or CD34 selection in both allogeneic [24,25] and autologous hematopoietic SCT after myeloablative conditioning has shown a notable increase in CMV disease and CMV-associated deaths [26]. Investigation of the effect of the source of stem cells on CMV infection provides conflicting results. Emergence of CMV infection and disease after either unmodified peripheral blood SCT or bone marrow transplantation was evaluated in 2 studies. One nonrandomized study (n = 158) showed that the incidences of CMV antigenemia (P = .01) and CMV interstitial pneumonia (P = .04) were significantly reduced in the peripheral blood SCT recipients compared with the bone marrow transplant recipients [26]. However, results from a randomized trial (n = 172) showed a higher incidence of CMV infection (P = .04) and a trend toward more CMV disease (P = .06) in unmodified peripheral blood SCT recipients when compared with bone marrow transplant recipients [27].

Table 1. *Results of Selected Studies of Cytomegalovirus (CMV) Serostatus and Patient Outcomes*

Study	No. Patients	T-Cell Depletion	Effect of CMV Serostatus on Mortality	Specific Outcomes
Broers [13]	115	Yes (n = 109) No (n = 6)	Yes (recipient)	5-y survival significantly better for CMV-seronegative than CMV-seropositive recipients, primarily because of treatment-related mortality: 64% versus 40% (P = .01)
McGlave [21]	1423	Yes	Yes (recipient)	CMV-seronegative recipients had a lower risk of death or relapse (hazard ratio, 0.80; P = .02)
Cornelissen [20]	127	Yes	Yes (recipient)	CMV-seronegative recipients had longer disease-free survival (P = .05) and lower transplant-related mortality (P = .08)
Craddock [15]	106	Yes	Yes (recipient)	5-y survival significantly better for CMV-seronegative than for CMV-seropositive recipients: 60% versus 42% (P = .006)
Kroger [19]	125	Yes	Yes (recipient)	CMV-positive serostatus of recipient associated with higher mortality (P = .014) and transplant-related mortality (P = .02)
Ljungman [16]	7018	No	Yes (donor)*	For HLA-identical siblings, donor CMV serostatus had no effect on outcomes in a univariate analysis For unrelated donor transplants, recipients of CMV-seropositive donor transplants had significantly improved survival (P = .006) and treatment-related mortality (P < .001) compared with recipients of CMV-seronegative donor transplants
Castro-Malaspina [14]	510	Yes (n = 389) No (n = 121)	Yes (recipient)	CMV-negative recipient serostatus was associated with significantly better overall survival (P = .002), reduced treatment-related mortality (P = .004), and higher cancerfree survival (P = .001)
Meijer [17]	253	Yes	Yes (recipient)	For recipients from matched, related donors, CMV seropositivity of either donor or recipient was not associated with survival or treatment-related mortality For recipients from matched, unrelated donors, recipient CMV seropositivity was associated with poorer survival (P = .013) and treatment-related mortality (P = .0070)
Nichols [18]	1750	No	Yes (recipient, donor)	Highest mortality associated with CMV-seropositive donor and CMV-seronegative recipient paining (P = .04) or CMV-seropositive donor and CMV-seropositive recipient paining (P = .03)

*This study examined the impact of donor CMV status among seropositive recipients. Effect of recipient serostatus was not evaluated.

Effect of the Conditioning Regimen

Data are accumulating on the effect of the pretransplantation conditioning regimen on CMV incidence and outcome. Myeloablative conditioning regimens seek to destroy the existing cells, allowing for replacement with the graft cells. With nonmyeloablative regimens, a mixed chimerism occurs, with coexistence of host and donor cells until the replacement graft material prevails. Because myeloablative conditioning regimens destroy host T cells (including the cytotoxic T cells specific for CMV), the question has been raised whether nonmyeloablative regimens would result in less early CMV infection, a shorter duration of neutropenia, reduced regimen-related toxicity, and a longer duration of host immunocompetence when compared with myeloablative regimens. To date, several nonmyeloablative and reduced-toxicity conditioning regimens have been reported (Figure 2), although the corresponding level of immunosuppression varies. In a study of mostly HLA-matched sibling transplant recipients with hematologic malignancies undergoing either nonmyeloablative (total body irradiation; 2 Gy) or conventional hematopoietic SCT, CMV disease was significantly delayed in the nonmyeloablative group compared with the myeloablative group (P = .02) [28]. However, the overall incidence of CMV disease at 1 year was similar between the 2 conditioning regimens in recipients at high risk (Figure 3). Notably, the incidence and onset

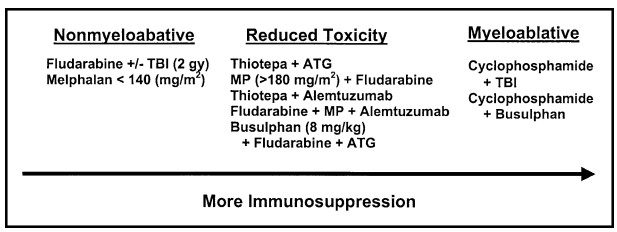

Figure 2. Continuum of intensity from nonmyeloablative to myeloablative hematopoietic stem cell transplant-conditioning regimens. ATG indicates antithymocyte globulin; TBI, total body irradiation; MP, melphalan.

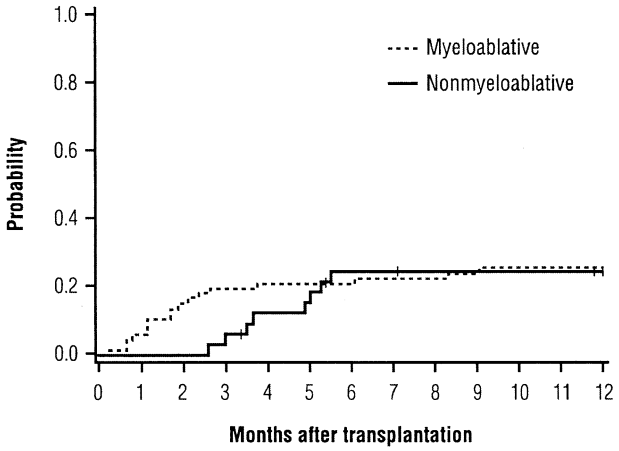

Figure 3. Incidence of cytomegalovirus disease in high-risk hematopoietic stem cell transplant recipients after nonmyeloablative and myeloablative conditioning regimens. Incidence at day 100, $P = .08$; incidence at day 365, $P = 87$; day of onset, $P = .02$. Reprinted with permission [28].

of CMV antigenemia, as well as the time to antigenemia clearance, were also comparable [28]. There was a trend toward more CMV infection and disease arising in unrelated donor transplant recipients [29]. However, a reduced-toxicity conditioning regimen of alemtuzumab-1H (immunoglobulin G1 humanized monoclonal anti-CD52) showed a high incidence of CMV infection with very early reactivation of virus in 50% of patients at a median of 27 days after engraftment [30]. The results of these studies suggest that the choice of conditioning regimens affects the time of CMV reactivation and disease, but the overall risks have not been substantially reduced in the nonmyeloablative SCT recipient.

Effect of Posttransplantation Immunosuppression

The immunosuppressive regimens that allow the recipient to retain the graft and avoid the complications of GVHD also play a role in CMV epidemiology. The net state of immunosuppression experienced by the recipient is modulated by factors such as pharmacologic therapies (type, timing, duration, and sequence), immunogenetic characteristics (HLA match), the presence or absence of immunomodulating viruses, and metabolic abnormalities [31]. Hematopoietic SCT recipients treated with high-dose corticosteroids (>1 mg/kg/d), mycophenolate mofetil, T cell–depleted autografts or allografts, and certain anti–T-cell strategies (eg, antithymocyte globulin) are considered at high risk for CMV disease. In one study, an increased risk of CMV disease or CMV-related complications in allogeneic stem cell recipients was associated with mycophenolate mofetil treatment, which seems to upregulate CMV [5]. In another study of allogeneic hematopoietic SCT recipients receiving intense immunosuppression and preemptive therapy, based on 2 positive DNA polymerase chain reaction (PCR) assays, the strategy failed because of the rapid rate of viral load increase [32]. In this population, treatment at a lower viral threshold is necessary and should not be delayed to obtain a second positive diagnostic test.

What emerges from these studies is that highly immunosuppressed recipients have delayed or reduced immune reconstitution, which has a direct effect on the viral replication dynamics in vivo [33]. Contrary to widely held beliefs that CMV is a slowly replicating virus (based on its time to clinical manifestations and its slow growth in fibroblast tissue cultures), the in vivo increase of viral load progresses with a doubling time of approximately 1 day in SCT recipients. The likelihood of subsequent CMV disease development is independently predicted by both the initial viral burden and the rate of viral burden increase. Therefore, measurement of these viral characteristics supports identification of recipients at immediate risk of CMV disease [33,34]. Overall, recipients with a higher viral burden are more prone to manifest CMV disease during both the early and the late transplantation periods [7,35]. The time frame for progression from viral detection to overt disease with a rapidly increasing viral load is compressed in highly immunosuppressed patients (eg, those taking >1 mg/kg/d of corticosteroids or those who are T-cell depleted) such that any positive test should trigger immediate treatment in these patients.

The same factors influence the viral load kinetics in patients receiving antiviral treatment. Nichols et al. [36] showed that viral load may increase in highly immunosuppressed individuals receiving ganciclovir. These viral load increases were seen in patients receiving high doses of corticosteroids.

LATE CMV DISEASE IN THE ERA OF PREEMPTIVE THERAPY

Although preemptive ganciclovir therapy administered in response to pp65 antigenemia [8] or PCR detection of CMV DNA [9] has dramatically reduced early CMV disease after allogeneic hematopoietic SCT, with concurrent improved survival in certain high-risk recipients [9,37], the resulting increase in the occurrence of late CMV disease has emerged as a threat to long-term survival. A recent prospective study explored the incidence and risk factors for late CMV infection in seropositive allogeneic hematopoietic SCT recipients receiving ganciclovir prophylaxis either at engraftment or preemptively in response to pp65 antigenemia, where routine antiviral drug use terminated after 3 months (Figure 4) [7]. Of the 146 patients followed up, 17.8% developed late CMV disease at a median of 169 days after engraftment. CMV disease was associated not only with a mortality rate of 46%, but also with recurrence in 38% of survivors [7]. Risk factors for late mortality and CMV disease are

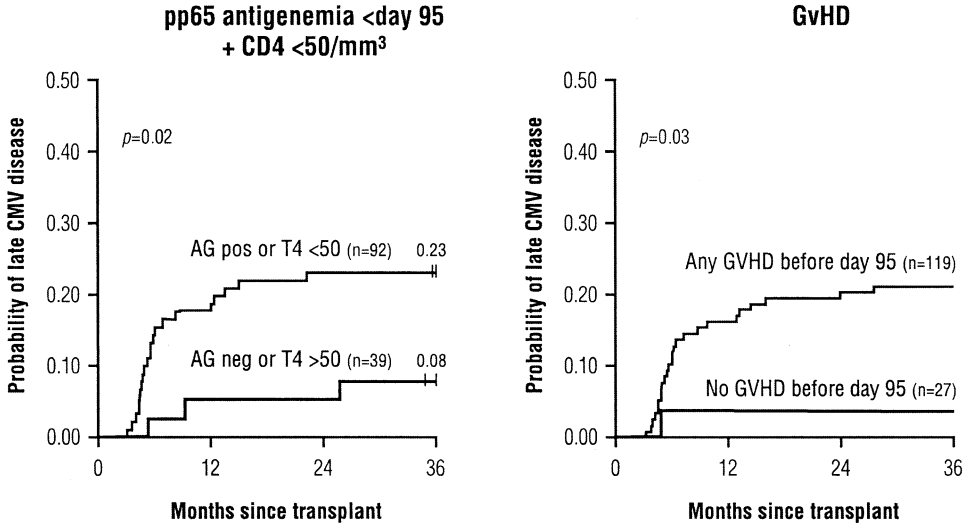

Figure 4. Cumulative incidence of late CMV disease in patients with the presence and absence of risk factors present at 3 months. Right, graft-versus-host disease (GVHD; grade 2-4 or clinical chronic). Left: Any pp65 antigenemia (AG) before day 95 and CD4 count <50 cells per cubic millimeter. Reprinted with permission [7].

shown in Table 2. Other studies have confirmed that chronic GVHD ($P = .0017$) and prior use of antiviral therapy for longer than 4 weeks ($P = .0073$) are risk factors for late CMV disease [10]. The authors of both studies recommended continued monitoring for CMV with rapid, sensitive techniques and prompt application of preemptive therapy, especially for high-risk recipients, in the late period after hematopoietic SCT.

Practically, the effectiveness of the preemptive strategy depends on compliance with testing. Although no randomized trials have been performed, cohort studies suggest that viral monitoring should occur weekly [28,38]. Thus, the logistics of frequent testing must be arranged with the patient (especially in the rural community setting). Although this has not been well studied, the frequency of monitoring may be reduced in patients receiving low doses of immunosuppression (ie, tapering doses of corticosteroids at <0.5 mg/kg/d) and in those without evidence of viral reactivation for 4 weeks [7] (Boeckh and Nichols, 2003, unpublished data).

CLINICAL MANIFESTATIONS OF CMV DISEASE

The direct clinical manifestations of CMV disease vary slightly for early compared with late disease. The most common manifestations are pneumonia and gastrointestinal disease, which can occur from the esophagus to the colon [39]. After day 100, manifestations

Table 2. *Risk Factors Associated with Late CMV Disease and Mortality in High-Risk Allogeneic Hematopoietic Stem Cell Transplant Recipients**

Variable	Relative Risk (95% Confidence Interval)	P Value
Risk factors present by month 3 after transplantation associated with mortality†		
Absolute lymphocytopenia after day 40 (≤100 cells/mm³)	1.8 (1.1-3.0)	≤.05
CD4 count (≤50 cells/mm³)	1.9 (1.1-3.1)	≤.05
CD8 count (≤50 cells/mm³)	2.5 (1.5-4.2)	≤.01
CMV pp65 antigenemia (any level positive)	1.8 (1.0-3.1)	≤.01
Surveillance risk factors present after day 95 after transplantation associated with late CMV disease‡		
Lymphocytopenia (<300 cells/mm³)	9.4 (3.8-23.5)	≤.01
CMV DNA (plasma)		
>1000 copies/mL	6.2 (1.0-39.2)	<.05
>10 000 copies/mL	12.3 (1.8-85.1)	≤.01
CMV pp65 antigenemia (any level positive)	5.3 (1.5-19.1)	≤.01
Risk factors present after day 95 after transplantation associated with mortality‡		
CMV disease	2.3 (1.2-4.2)	≤.01
Lymphocytopenia (<300 cells/mm³)	3.6 (1.9-6.7)	≤.01

*Adapted with permission [7].
†Univariate analysis.
‡Multivariate analysis.

other than pneumonia and gastrointestinal disease are sometimes observed, including CMV retinitis [40,41] and central nervous system disease [13,42]. Standardization of definitions for use in clinical studies of CMV infection and disease in immunocompromised patients has allowed more accurate assessments [43].

The indirect effects associated with CMV include graft rejection, accelerated atherosclerosis, and bacterial or fungal superinfections; these have been primarily documented in solid organ transplantation [44-47]. Early studies with acyclovir prophylaxis for CMV infection after bone marrow transplantation provided preliminary indications of indirect CMV effects [48]. More recently, the indirect effects of primary CMV infection were implicated in the higher incidence of bacterial and fungal infection in a large cohort of seronegative recipients of hematopoietic SCTs from seropositive donors [18]. Efforts to elucidate and ameliorate these indirect effects have stimulated an impressive body of research from clinical trials, as well as basic research. Inclusion of indirect effects as valuable end points (especially in clinical trials of drug therapies) should help assess these effects [31,43].

CMV INFECTION DIAGNOSIS AND SURVEILLANCE

CMV disseminates through the blood during active infection, and the presence of viremia is recognized as the major virologic risk factor for progression to clinical disease. Older techniques, such as culture-based assays, failed to provide the rapid, sensitive, and efficient detection and quantitation required for prediction of outcomes or initiation (or changes) of antiviral treatments [3]. In the era of preemptive antiviral treatment strategies, the most universally applicable clinical assays are CMV DNA detection methods and the pp65 antigenemia assay. Recently, the pp67 messenger RNA (mRNA) assay has been shown to be an alternative to these methods [49]. In general, use of PCR technology with either in-house or commercially available assays to detect CMV DNA has proven an excellent guide to trigger preemptive therapy. However, the individual assays vary in sensitivity, specificity, and predictive value. Although plasma was long considered a poor source for PCR-driven assays, improvement of these assays through sophisticated technology provides sensitivity similar to that of cell-based sources [50,51]. Another practical issue concerns assay variability. All quantitative measurements have a certain coefficient of variation, so the general guideline for a true increase or decrease in viral load is 0.5 $\log_{10}$—approximately a 3-fold difference. Although the pp65 antigenemia assay, the pp67 mRNA assay, and most DNA PCR assays perform well in clinical practice [39] (Table 3), assay sensitivity and low variability of quantitation become especially critical for highly immunosuppressed recipients, in whom the viral load can increase rapidly [53]. For these recipients, monitoring by quantitative PCR with immediate antiviral therapy at low levels without waiting for a second positive test may affect CMV-related outcomes [8,32,54,55].

CURRENT PREVENTION AND TREATMENT STRATEGIES

Prevention of Primary CMV Infection

For CMV-seronegative hematopoietic SCT recipients, prevention focuses on use of seronegative donors and blood products or use of leukocyte-reduced blood products. However, the rate of CMV disease with leukocyte-reduced blood products can be up to 2.4% [56]. In a follow-up study of 807 patients receiving leukocyte-reduced or seronegative blood products, filtered red blood cell units (but not apheresis platelet products) from CMV-positive donors were associated with a significant 32% increase in the odds for transfusion-transmitted CMV ($P = .006$). These results underscore that CMV-seronegative products should remain a critical resource for high-risk patients [57]. Although posttransplant surveillance and preemptive therapy are currently not uniformly advocated [58,59], their use is highly effective in eliminating complications of transfusion-related CMV infection [57].

Prophylaxis Strategies

Immune Globulin and Antibody Therapy. The use of CMV-specific intravenous immune globulin (IVIG) for viral prophylaxis has been assessed in 2 separate randomized, controlled trials of treatment of seronegative recipients of allogeneic bone marrow transplants from seropositive donors, and these studies showed no difference in disease incidence [60,61]. Other studies of IVIG or CMV immunoglobulin have failed to show either consistent positive results for CMV-related complications or survival benefits [62-65]. A trial with a highly neutralizing monoclonal antibody (MSL-109) specific to the CMV glycoprotein H also failed to show a benefit in seropositive hematopoietic SCT recipients [66]. Overall, antibody treatments are not currently recommended for CMV prophylaxis [58], and uncertainty remains over the usefulness of IVIG or hyperimmune globulin for the prevention of non-CMV complications.

Acyclovir and Valacyclovir. The first trial of antiviral prophylaxis used acyclovir (500 mg/m^2) intravenously (IV) every 8 hours from 5 days before engraftment to 30 days after transplantation in seropositive allogeneic bone marrow transplant recipients [67]. This prospective, nonrandomized study showed that high-dose acyclovir therapy reduced the risk of CMV infection and invasive disease, as well as mortality, in the first

Table 3. *Results of PCR-, pp67 mRNA–, and pp65 Antigenemia-Guided Preemptive Treatment Studies**

Study**	Surveillance	Control Group	Treatment Regimen (Start/End)	N	Incidence of CMV Disease (day 100) (%)
Randomized trials					
Einsele [9]	PCR CMV DNA	Rapid culture-guided ganciclovir	Ganciclovir and CMV Ig for 2 consecutive positive PCR results; discontinuation after 2 wk or when PCR negative; repeated treatment if PCR positivity recurred	26†	7.7
Boeckh [8]	pp65 AG	Ganciclovir prophylaxis	Ganciclovir for pp65 AG (≥2 positive cells per 150 000 PBL; discontinuation after 3 wk or 6 d after negative pp65 AG assay; repeated treatment if positive AG recurred	114†‡	14.1§
Moretti [109]	pp65 AG	Preemptive foscarnet or ganciclovir	Ganciclovir or foscarnet for 15 d for pp65 AG (1 to 4 positive cells per 200 000 PBL)	19‖	10.5
Humar [110]	pp65 AG	Day 35 BAL	Positive pp65 AG: ganciclovir 5 mg/kg twice daily for at least 2 wk or until negative AG; repeated treatment if necessary Positive BAL: ganciclovir 5 mg/kg twice daily for 2 wk followed by 8 wk of 5 mg/kg/d, 5 d/wk	60	0
Reusser [78]	pp65 AG, PCR CMV DNA	Preemptive ganciclovir or foscarnet for positive AG or PCR	Ganciclovir: 14 d of 5 mg/kg twice daily Foscarnet: 14 d of 60 mg/kg twice daily Continued maintenance therapy if persistent positive screening test at 2 wk	110 (FSC) 103 (GCV)	3.3 (both groups combined)¶
Gerna [49]	pp67 mRNA	pp65 AG	Ganciclovir 5 mg/kg twice daily until 2 negative tests	41 (pp67 mRNA)#	0 (both groups)
Nonrandomized trials					
Ljungman [89]	PCR CMV DNA	Historical	Ganciclovir for 2 consecutive positive results DNA	39 (pp65 AG)# 58	6.0
Gerna [111]	pp65 AG	None	Ganciclovir for pp65 AG (≥2 positive cells per 200 000 PBL); discontinuation after 2 wk; maintenance for 7 to 14 d in patients with GVHD; repeated treatment if pp65 AG recurred	30#	6.6
Boeckh [112]	pp65 AG	Historical	Ganciclovir for pp65 AG at any level until day 100	102‡	3.8
Einsele [10]	PCR CMV DNA	None	Ganciclovir for 2 consecutive positive PCR results for 14 d; if still positive, foscarnet until PCR negative	86	3.5

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CMV indicates cytomegalovirus; PCR, polymerase chain reaction; Ig, immunoglobulin; AG, antigenemia; PBL, peripheral blood leukocyte; BAL, bronchoaveolar lavage; FSC, foscarnet; GCV, ganciclovir; GVHD, graft-versus-host disease.

*Data refer to the incidence of CMV disease in seropositive recipients or seronegative recipients with seropositive donors.

†Incidence among engrafted patients.

‡Study includes only seropositive recipients.

§Disease before or after AG of 2 positive cells per slide, 8.8%; disease shortly after discontinuation of ganciclovir on the basis of a negative test, 5.3%.

‖Only disease after AG was reported; 1 patient was seronegative with a seronegative donor.

¶There was no difference in the incidence of CMV disease between the 2 groups. Incidence figures refer to the time after the start of study drug rather than the entire first 100 days.

#Pediatric patients only.

**Additional studies have been reported without data provided for day 100 [27,52].

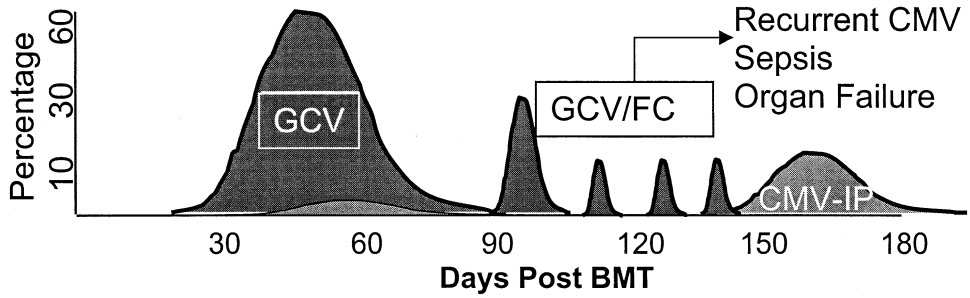

Figure 5. Approximate time of CMV infection after allogeneic hematopoietic stem cell transplantation during the era of preemptive treatment strategies with ganciclovir. GCV indicates ganciclovir; FSC, foscarnet; IP, interstitial pneumonia; BMT, bone marrow transplantation.

100 days after transplantation. With the apparent success of acyclovir, a more extensive prospective, double-blind trial showed a survival advantage for the IV acyclovir/oral acyclovir group compared with the controls (low-dose oral acyclovir/placebo group). There was no difference in the incidence of CMV pneumonia or other CMV diseases between the treatment groups. The survival benefit of 19% was maintained through 1 year of follow-up. In addition, there seemed to be fewer deaths related to bacterial and fungal infections [48,68].

The development of a valine-ester prodrug of acyclovir, valacyclovir, allowed investigators to explore 2 issues limiting acyclovir use as CMV prophylaxis: (1) poor oral bioavailability of the parent acyclovir and (2) potential to expand dosage and the duration of treatment with an improved oral formulation. One large study randomized 727 seropositive (recipient-positive or donor-positive) allogeneic bone marrow transplant recipients to either oral valacyclovir (2000 mg) or oral acyclovir (800 mg) 4 times per day for 18 weeks after transplantation after prophylactic IV acyclovir therapy (500 mg/m^2 IV every 8 hours from 5 days before engraftment to 28 days after transplantation) [69]. Treatment with valacyclovir significantly reduced the risk of CMV viremia compared with high-dose oral acyclovir (28% versus 40%, respectively; $P < .0001$) during the approximately 160 days of follow-up. Preemptive treatment with ganciclovir guided by either PCR or antigenemia detection of CMV resulted in a low incidence of CMV disease in the valacyclovir group (3.6%) and in the acyclovir group (5.6%). Survival did not differ between treatment groups. Tolerability was also similar for the 2 drugs [69]. In a subset analysis, the effect of valacyclovir was seen mainly in low-risk patients, whereas little effect was shown in recipients of unrelated donor grafts. A small randomized trial of valacylovir versus intravenous ganciclovir showed no statistically significant difference in CMV disease, neutropenia, and survival [70].

Ganciclovir. Results of early prophylaxis with ganciclovir in seropositive allogeneic bone marrow transplant recipients have been reported from 3 randomized, double-blind studies with slightly different protocols [8,71,72]. Although all studies demonstrated a significant ($P < .001$) reduction of CMV infection or disease during the first 100 days after engraftment, 2 studies also showed a significant ($P < .001$ and $P = .002$, respectively) and near-total prevention of CMV disease during ganciclovir therapy [8,70]. No survival advantage was demonstrated, and severe neutropenia was observed in all studies and was potentially linked to greater risks of fungal or bacterial infections [8,71,72]. However, none of these studies had a sufficiently large sample size to evaluate survival as a study end point. Thus, there was no detectable survival benefit with early prophylaxis in these 3 studies, whereas analyses of large high-risk cohorts, such as unrelated donor transplant recipients [37] and randomized studies of preemptive therapy performed in high-risk individuals, did show an effect of ganciclovir on survival [9,73]. Studies listed in Table 1 indicate that there are now important subsets of patients in whom the use of ganciclovir prophylaxis or preemptive therapy leads to the elimination of the survival disadvantage associated with pretransplantation CMV serostatus.

Foscarnet. Only small uncontrolled studies of foscarnet in hematopoietic SCT recipients have been conducted for CMV prophylaxis. Renal toxicity has limited widespread prophylactic use of this agent [74,75].

Preemptive Strategies

Preemptive therapy in hematopoietic SCT recipients refers to identification of at-risk recipients by using timely CMV detection with PCR techniques or with quantitative pp65 antigenemia, followed by immediate implementation of antiviral treatment on viral detection (Figure 5). The premises of preemptive therapy are that CMV viremia predicts disease development and that viral load is a critical factor in pathogenesis [3,35]. Therefore, this approach requires access to reliable and rapid early diagnostic tests, strict monitoring (at least weekly), and appropriate sample selection [3]. Another risk-adapted strategy (sometimes called *selected prophylaxis*) uses immunologic and

virologic risk factors (eg, antithymocyte globulin therapy, CMV antigenemia, or GVHD) to adjudicate administration of a short course of ganciclovir. However, although this is reasonable in high-risk situations, studies on this approach are sparse, and general applicability remains untested [76,77].

Ganciclovir and Foscarnet. Currently, preemptive therapy with IV ganciclovir has decreased the cumulative incidence of CMV disease to rates ranging from 3.3% to 10.5% before day 100 after engraftment (Table 3). Although most data are available for ganciclovir regimens, both ganciclovir and foscarnet are used in preemptive therapy. In a large randomized trial of 213 SCT recipients, preemptive therapy with foscarnet was compared with that with ganciclovir; the incidence of CMV disease was <5% in both groups [78]. Neutropenia was more frequently reported in the ganciclovir arm, whereas impaired renal function and electrolyte abnormalities were more frequent in the foscarnet arm [78]. Despite the higher incidence of neutropenia, the composite end point of CMV disease–free survival was not different between the groups. A small study of reduced-dose ganciclovir and foscarnet combinations did not show a reduction in viral load or treatment-related toxicities [79].

Oral Ganciclovir and Valganciclovir. Pharmacokinetic evaluations of an oral ganciclovir formulation in a phase I/II study showed that the presence of acute GVHD of the gastrointestinal tract did not seem to limit drug absorption, and, thus, the bioavailability of the drug seemed appropriate for hematopoietic SCT recipients. Gastrointestinal intolerance and neutropenia in the early postengraftment period (before day 60) seemed to be impediments to the use of this formulation [80]. Use of the oral ganciclovir formulation later in the transplantation course after IV treatment provided acceptable tolerability and efficacy in another study [81].

Valganciclovir is a valine-ester prodrug of ganciclovir that, in solid organ transplant recipients [82] and human immunodeficiency virus–infected patients [83], has improved bioavailability, resulting in an area under the curve similar to that of IV ganciclovir. If the early reports of drug usefulness are supported by currently ongoing clinical trials in hematopoietic SCT, valganciclovir may become an alternative to IV ganciclovir for some indications [84,85].

Cidofovir. Although randomized clinical trials have not yet been performed, small, uncontrolled studies of cidofovir in allogeneic hematopoietic SCT recipients indicate that it may be effective as second-line therapy in patients with CMV disease whose traditional antiviral treatments have failed [86] or in low-risk patients after low-intensity conditioning regimens [87]. However, treatment-related toxicities (ie, renal and marrow impairments) and reports of cidofovir failures prohibit its use as front-line preemptive therapy before comparative studies are conducted [86-88].

Initiation and Duration of Preemptive Therapy and Viral Monitoring. The definitive threshold for triggering antiviral preemptive therapy remains undecided, and centers continue to base their regimens primarily on local experience and patient characteristics. In general, initiation of therapy triggered by 2 consecutive positive results with either DNA PCR-guided or any positive pp65 antigenemia test result is effective in allogeneic recipients receiving standard immunosuppression [9,54]. In cases of highly immunosuppressed recipients, the faster increase in CMV levels warrants therapy initiation at very low levels of virus detection [32].

Various durations of antiviral treatment within preemptive therapy have been explored. The original studies continued any initiated ganciclovir therapy until day 100 after engraftment (approximately 6 to 8 weeks in the average recipient). Studies from the mid 1990s of shorter ganciclovir courses based on negative PCR assays at the end of therapy with a 3-week mean [9] or a 2-week mean [89] were generally effective; however, resumption of preemptive therapy was necessary in approximately 30% of patients (Figure 5). Most centers now continue antiviral treatment until the designated viral marker is negative. If less sensitive markers, such as the pp65 antigenemia assay or pp67 mRNA assay, are used, then preemptive therapy should be continued until 2 negative assays are obtained [69]. Induction dosing should continue until a decline of viral load has been demonstrated [55].

If either the recipient or donor is seropositive, weekly CMV surveillance is recommended until day 100 after engraftment. After day 100, weekly surveillance should be continued in recipients at high risk for disease on the basis of early patient characteristics [39] (Table 4). Because 80% of CMV cases are documented before day 270 after engraftment [7], surveillance should be performed during this period; the duration and frequency of CMV monitoring in the later transplantation periods have not been determined. Nonetheless, a surveillance strategy should include considerations of the net immunosuppression of the recipient (including corticosteroid treatments for GVHD) and history of CMV reactivation. Thus, selected patients with continued intense immunosuppression may benefit from surveillance after day 270 after transplantation.

EFFECT OF DRUG-RESISTANT CMV STRAINS

Overall, ganciclovir resistance remains a rare occurrence in hematopoietic SCT recipients [36,42,90-93] (Table 5). Most reports of ganciclovir resistance in transplant recipients suggest that children with immu-

Table 4. *Management of CMV Infection after Hematopoietic Stem Cell Transplantation: Recommendations [39]*

Indication	Strategy	Comment
Prevention		
Allogeneic transplantation (myeloablative and nonmyeloablative)	Antigenemia- or PCR-guided early ganciclovir treatment: 5 mg/kg BID for minimum of 7 d or until viral load decreases (whichever comes later), followed by 5 mg/kg/d until day 100 or until serial negative PCR or antigenemia* *or*	Some cases of CMV disease may occur shortly after discontinuation based on negative PCR or antigenemia; thus, 2 negative tests are required in high-risk settings
Seropositive recipient	Ganciclovir prophylaxis at engraftment: 5 mg/kg BID for 5 d followed by 5 mg/kg/d on 5-6 dy/wk until day 100	Recommended if neither PCR no antigenemia testing is available
Seronegative recipient/ seropositive donor	Antigenemia- or PCR-guided early ganciclovir treatment: 5 mg/kg BID for 7 d or until viral load decreases, followed by 5 mg/kg/d until day 100 (or until negative PCR or antigenemia)* *and* Seronegative or leukocyte-reduced blood products	Prophylaxis at engraftment not recommended because of low incidence of posttransplantation infection
Seronegative recipient/ seronegative donor	Seronegative or leukocyte-reduced blood products	Breakthrough disease of up to 2.3%; virologic monitoring and preemptive therapy effective in reducing breakthrough rates but not uniformly advocated
Autologous transplantation, seropositive recipient	Antigenemia- or PCR-guided early ganciclovir treatment: 5 mg/kg ganciclovir BID for 7 d or until viral load decreases, followed by 5 mg/kg/d for 14 to 21 d	Monitoring not uniformly advocated because of very low risk in some settings (absence of corticosteroids, TBI, CD34 selection); CD34-depleted recipients represent a special population that should be monitored similarly to allogeneic recipients
Seronegative recipient	Seronegative or leukocyte-reduced blood products	Breakthrough disease of up to 2.3%; virologic monitoring and preemptive therapy effective in reducing breakthrough rates but not uniformly advocated
Treatment of disease		
CMV pneumonia	Ganciclovir 5 mg/kg BID for 14 to 21 d (or until declining viral load) followed by 5 mg/kg/d for at least 3 to 4 wk plus IVIG 500 mg/kg or CMV Ig 150 mg/kg every other day for 2 wk, then weekly	Extended maintenance throughout the period of severe immunosuppression (ie, GVHD treatment) may be considered
Gastrointestinal disease	Ganciclovir 5 mg/kg BID for 14 to 21 d (or until declining viral load) followed by 5 mg/kg/d for at least 3 to 4 wk	If deep ulcerations are present, longer maintenance may be required
Marrow failure	Foscarnet 90 mg/kg BID for 14 d followed by 90 mg/kg/d for 2 wk plus G-CSF	Ganciclovir plus IVIG has also been used
Retinitis	Ganciclovir 5 mg/kg BID for 14 to 21 d followed by 5 mg/kg/d for at least 3 to 4 wk	Extended maintenance may be required; ophthalmologic monitoring is required

TBI indicates total body irradiation; IVIG, intravenous immune globulin; Ig, immunoglobulin; G-CSE, granulocyte colony-stimulating factor; BID, twice daily.

*Foscarnet has been shown to be equivalent with regard to efficacy in 1 randomized trial [78].

nodeficiency syndromes or those who receive transplants from haploidentical donors with T cell–depleted allografts are at higher risk for development of CMV strains resistant to ganciclovir (multidrug resistance to foscarnet and cidofovir also occur) [93]. Although children seem at higher risk in the early period (days 44 to 95 after allogeneic engraftment with T cell–depleted material) before recovery of immune competence, ganciclovir resistance does not always correlate with delayed immune recovery [94]. Ganciclovir resistance emerged in 2 adult haploidentical hematopoietic SCT recipients after prolonged preemptive therapy [42]. One case involved central nervous system manifestations linked to mixed wild-type and UL97 mutant viral populations in the cerebrospinal fluid. The second case resulted from a resistant variant in the lung [42]. The incidence of ganciclovir resistance in hematopoietic SCT continues to be monitored through clinical response assessments and identification of resistance-associated mutations.

Resistance to ganciclovir results from mutations in the viral DNA polymerase gene (UL54) and the viral

Table 5. *Studies Documenting Ganciclovir Resistance in Hematopoietic Stem Cell Transplant Recipients*

Study	Setting	N	Incidence (%)
CMV disease			
Slavin [90]	CMV interstitial pneumonia	12	8.3
CMV infection			
Erice [91]	CMV infection	15	6.7
Nichols [36]	pp65 antigenemia (all)	119	0.8
	Increasing pp65 antigenemia (subset)	15	6.7
Boivin [92]	pp65 antigenemia	34	0
All patients			
Eckle [93]	Unrelated donor/haploidentical	79	3.8
Wolf [42]	Allogeneic/haploidentical (all)	138	1.4
	Haploidentical (subset)	26	7.6

phosphotransferase gene (UL97), with mutations in UL97 emerging first (resulting in low-level resistance), followed by the more severe resistance associated with UL54 mutations [91,95]. The development of ganciclovir resistance is linked to the degree of immunosuppression, the kinetics of viral replication, the presence of mutant viral strains at the onset of drug therapy, and the effectiveness and duration of antiviral therapy. Situations in which prolonged subclinical reactivation occurs in the presence of antiviral drug predispose patients to development of drug resistance. With quantitative virologic monitoring, increases in viral load for >2 weeks on induction doses of antivirals may be suggestive of viral resistance. Although switching antiviral drug therapy to foscarnet or cidofovir (which are not affected by the UL97 mutation) has shown benefit, some cross-resistance has been reported [93,95,96]. Use of the prodrug valganciclovir in solid organ transplant recipients has suggested that lower rates of resistance may occur when compared with those seen with oral ganciclovir, but data are not yet available for hematopoietic SCT recipients [97].

FUTURE PHARMACOLOGIC AND IMMUNE THERAPIES

Clinical trials are under way with valganciclovir. The drug is well absorbed, with proven efficacy as induction therapy in human immunodeficiency virus-infected patients with new CMV retinitis, and preemptive studies are ongoing [84]. However, absorption in SCT recipients with gastrointestinal GVHD has not been studied. In hematopoietic SCT, randomized studies are ongoing to determine the role of valganciclovir in preemptive therapy and for long-term prophylaxis of late CMV infection and disease. Initial uncontrolled reports show no breakthrough disease in a small cohort of hematopoietic SCT recipients receiving preemptive therapy [98]. Additional drugs are in preclinical and clinical development [99, 100].

The profound and prolonged immunodeficiency engendered by the processes necessary for hematopoietic SCT provides the opportunity for CMV reactivation. Current immunotherapy approaches involve 2 strategies: (1) early restoration of recipient CMV-specific immunity with adoptive transfer of expanded T-cell clones from donor material [101-103] and (2) vaccination of stem cell donors with or without recipient vaccination. For the adoptive transfer approach, research interest has centered on restoration of CMV-specific $CD8^+$ cytotoxic T cells, because initial studies showed that these cells were capable of preventing the development of CMV-associated disease in the early postengraftment time frame [104]. More than a decade ago, the potential of adoptive transfer of CMV-specific $CD8^+$ T-cell clones propagated in vitro was reported in allogeneic hematopoietic SCT recipients [105]. More recently, adoptive transfer of cloned or expanded CMV-specific $CD8^+$ T cells from the blood of their CMV-seropositive donors was successfully accomplished in allogeneic hematopoietic SCT recipients, with resultant substantial increases in anti-CMV cytotoxic activity and no treatment-related side effects [101]. However, this study also highlighted the need for concurrent transfer of $CD4^+$ helper T cells. Another recent study reported infusion of CMV-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy [106].

A number of strategies to produce sufficient quantities of T cells appropriate for adoptive immunotherapy have been explored; major technical issues include maximizing comprehensive and efficient antigen presentation and selection of the T-cell subsets most likely to avoid the risk of GVHD. For expansion of T-cell clones through presentation of exogenously provided antigen, the choice of antigen-presenting cells includes monocytes, dendritic cells, and B-lymphocyte cell lines. Techniques for presentation of endogenously provided antigens have used a variety of antigen-presenting cells, transduction versus transfection approaches, different vectors, and different antigens. Approaches for selection of the most favorable lymphocyte subset involve boosting cell numbers and identifying and retrieving the cells [107]. Host factors and immunosuppressive or treatment-related drugs are likely to influence the success of immune reconstitution after hematopoietic SCT. In particular, use of high-dose corticosteroids (>1 mg/kg/d) has already been identified as predicting poorer recovery of competent T-cell immunity during the first 3 months after transplantation [108]. The major issues with clinical application of adoptive immunotherapy include technical and time-dependent challenges, lack of appropriate donor material, further characterization of host

and regimen effects, and the expense of this treatment approach.

Extensive literature exists on CMV-directed vaccines using material derived from attenuated live virus, recombinant live virus, viral DNA sequences, whole CMV proteins, and peptides. However, these approaches will be successful only in the recipient with adequate postengraftment immune reconstitution, especially for the CMV-specific T cells. Vaccine-based approaches for the immunosuppressed host are still hindered by the major obstacle of producing an effective vaccine based on products other than live virus. Even if such a vaccine were available, the best approach for its application remains to be defined. Should seropositive donors be boosted, or should seronegative donors be vaccinated, with or without subsequent stem cell recipient vaccination [103]? Although immunotherapy seems to be the most comprehensive approach in theory, practical clinical application requires more intensive investigation.

CONCLUSIONS

Although many of the problematic issues of CMV infection and disease in hematopoietic SCT recipients remain active areas of investigation, significant progress has been made in reducing the clinical effects of infection in recipients at low to average risk. However, the highly immunosuppressed recipient remains at high risk for infection and subsequent late CMV disease manifestations, as well as indirect effects of CMV. Major advances in prevention of CMV infection occurred with the introduction of the antiviral agent ganciclovir and the more widespread use of sophisticated viral diagnostics. These advances, combined with identification of at-risk recipients, resulted in effective application of the preemptive therapy approach to minimizing early viral infection. One surprising finding is that the preemptive approach, although highly effective in preventing CMV disease (Table 3), has not eliminated the survival disadvantage associated with CMV seropositivity in highly immunosuppressed patients, such as recipients of T cell–depleted grafts (Table 1). This may be due to indirect effects of CMV not covered by preemptive therapy; thus, prophylaxis using highly effective antivirals may be warranted in these situations. However, drug toxicity remains a major impediment with currently available drugs. New anti-CMV drugs with improved tolerability are needed. Meanwhile, prophylactic approaches with intensified supportive measures (eg, ganciclovir or valganciclovir with the administration of granulocyte colony-stimulating factor) may be studied to examine whether preventing the effects of neutropenia results in improved overall outcomes in high-risk CMV-seropositive recipients. The increased incidence of late CMV infection and disease is another practical concern, because many recipients are no longer under the care of the specialty oncology center when reactivation occurs. The emerging issue of drug-resistant viral mutations presents new challenges for successful patient outcomes. Efforts to eliminate the effects of CMV infection require the development of improved antiviral agents and basic research on more fundamental immunotherapy approaches.

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