Blood and Marrow TRANSPLANTATION

REVIEWS

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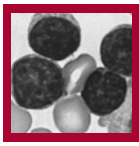

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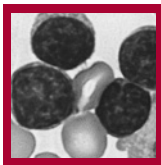
Cytomegalovirus: Down but Not Out

by John R. Wingard, MD, Editor

Once the most feared infectious pathogen threatening patients after hematopoietic cell transplantation, cytomegalovirus (CMV) today is merely a shadow of its former self. Introduction of effective antivirals, validation of bronchoalveolar lavage as a less invasive means of diagnosis of CMV pneumonia, development of rapid diagnostic methods for analyzing blood specimens to identify infection before onset of disease, and testing of prophylactic and preemptive strategies to prevent morbidity and mortality have all contributed to tame this bully. The battle is not ended, however. This organism continues to stage ambushes that necessitate continued vigilance.

In this transcript of a satellite symposium held at the annual Tandem BMT Meetings in Keystone, Colorado, January 30 to February 4, 2003, current management strategies and continuing clinical dilemmas are discussed. The first presentation reviews the progress made in prevention and treatment of CMV infection, the pros and cons of prophylaxis versus preemptive therapy, the factors that affect the choice of drugs and the length of treatment, and the growing concern of late-onset CMV disease. The second presentation discusses how to choose the best diagnostic test and discusses the risk for emergence of drug resistance. The third presentation focuses on late-onset CMV disease: risk factors and strategies for prevention and preemptive therapy.

Truly we have come a long way in the past two decades. As with suppression of early infection and disease caused by other herpesviruses, forced patience in CMV allows resurgence later in patients whose immunity remains weakened. There is yet work to be done.



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American Society for Blood and Marrow Transplantation

Executive Office
American Society for Blood and Marrow Transplantation
85 West Algonquin Road, Suite 550
Arlington Heights, IL 60005-4425
(847) 427-0224; fax (847) 427-9656
e-mail: mail@asbmt.org

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Symposium Report

Blood and Marrow TRANSPLANTATION REVIEWS

Adapted from a symposium funded by an unrestricted educational grant from Roche Laboratories, Inc.

Impact of CMV in Stem Cell Transplantation

2003 Tandem BMT Meetings

January 30, 2003, Keystone, Colorado, USA

John Wingard,[a] Michael Boeckh,[b] Garrett Nichols[b]

[a]Bone Marrow Transplant Program, University of Florida College of Medicine, Gainesville, Florida;
[b]Program in Infectious Disease, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Current Treatment and Prevention Options for CMV

John Wingard, MD

Cytomegalovirus (CMV) has been an enormous threat to bone marrow transplant (BMT) recipients and has represented a major cause of morbidity and mortality after bone marrow transplantation.

The typical scenario of CMV disease in transplantation patients has been a patient who engrafts after an allograft and then develops graft-versus-host disease (GVHD) that is controlled with corticosteroids. The patient is discharged to the outpatient clinic, and several weeks later presents in the clinic with a nonproductive cough and is found to have low-grade fever and a chest radiograph showing some mixed interstitial alveolar infiltrates (Figure 1). These patients very quickly become hypoxic and within 2 weeks are on the ventilator. Death occurs 85% of the time. Historically the median onset of CMV disease has been in the 2nd to 3rd month after transplantation, with a median onset time of 50 days. Ninety percent of cases have occurred within the first 3 months posttransplantation. This clinical syndrome of pneumonia is the most common presentation, although in recent decades an increasing incidence of enteritis has also been noted. CMV infection accounts for one half of all cases of interstitial pneumonitis, the other half being idiopathic syndrome presumably due to the conditioning regimen. As I mentioned, the case fatality rate has been exceedingly high and for many years has represented the leading cause of infectious death in transplantation patients.

Risk Factors for CMV Disease

Not all patients are equally vulnerable to this deadly complication. A number of risk factors have been noted in observational studies over the last several decades (Table 1). Host factors, particularly older age, have been noted in various series.

A number of transplantation factors have also been noted. Allograft recipients are at substantially greater risk than autograft recipients. Patients who have received intensive conditioning regimens also are more vulnerable, as are recipients of transplants from unrelated donors or alternate donors for which there is mismatching between the donor and the recipient. Increased susceptibility occurs in patients who have undergone T-cell depletion techniques, either ex vivo or in vivo through the use of purine analogs or monoclonal antibodies directed against T-cells. The occurrence of GVHD in which immune responses are dysregulated or in which protective immune responses are impaired is also a risk factor.

Certain viral factors are also very important, and it was very quickly noted that patients who were CMV seropositive prior to receiving a transplant were at substantially greater risk than seronegative patients. Bear in mind that CMV is a latent virus that is endogenous, a characteristic that presumably is contributory to many of these episodes. Patients who develop viremia detected by either older cultural techniques or polymerase chain reaction (PCR) or CMV antigen techniques, those with higher burdens of virus load, are more susceptible than those with lower virus load.

Immune factors are also observed to be, very important. Protective cytotoxic T-cell responses are critical not only for the prevention of the occurrence of the disease but also for recovery from disease once it occurs. For many years, we overlooked the issue of CMV infection in autograft recipients, but over the last decade it has become increasingly clear that these individuals are also vulnerable,

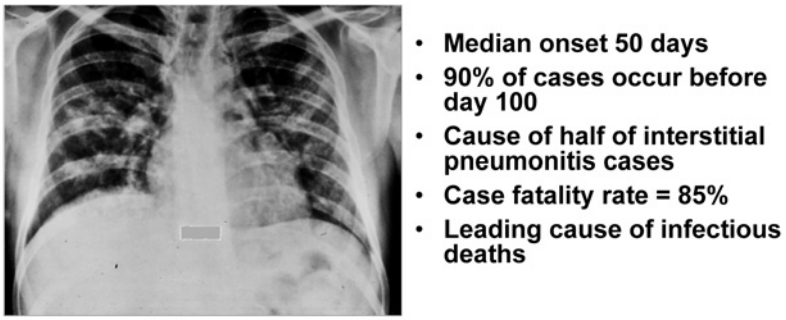

Figure 1. Chest radiograph showing some mixed interstitial alveolar infiltrates typically found in cytomegalovirus pneumonitis patients.

Table 1. Risk Factors for CMV Disease after Allogeneic Hematopoietic Stem Cell Transplantation

- Host
  - Older age
- Transplantation
  - Allogeneic BMT
  - Intensive conditioning regimen
  - Unrelated or mismatched donor source
  - T-cell depletion (ex vivo or in vivo)
  - GVHD
- Viral
  - Seropositivity
  - Viremia
  - High viral burden
- Immune
  - Lack of cytotoxic cellular responses

although at a substantially lower rate. But again certain factors can increase vulnerability in autograft recipients. Certainly patients receiving more intensive conditioning regimens, particularly those with hematologic malignancies, are more vulnerable, and those who have had prior therapy as well as peritransplantation therapies, particularly using the purine analogs, fludarabine, cladribine, and pentostatin. These treatments are increasingly being used in the nontransplantation setting. Many of our autotransplant recipients are coming to us substantially more immunosuppressed than in decades earlier. Likewise patients who are receiving anti–B-cell or T-cell monoclonal antibodies such as rituximab or alemtuzemab either before transplantation or in the peritransplantation period are more vulnerable. The use of corticosteroids, either before or during the transplantation period, similarly exacerbates the vulnerability. CD34 selection, which removes many immune cells from the autograft, also increases the vulnerability, and indeed individuals undergoing this procedure often are also concomitantly receiving steroids or other purine analogs or antibody therapies that can lend vulnerability. In one series in Seattle, in which several of these factors were present, 23% of the autograft recipients who had CD34-selected grafts with concomitant other medications were susceptible to CMV disease.

Advances in Diagnosis and Treatment of CMV Disease

CMV morbidity and mortality present a sobering picture indeed, but thankfully enormous strides have been made to lessen the threat from this potentially deadly pathogen (Table 2). Observational studies to identify those most vulnerable are key in identifying strategic opportunities for controlling this potentially deadly infection, and a series of treatment advances have made possible enormous improvements in patient outcome.

One of the first diagnostic advances was an improved culture technique. Formerly it took weeks to identify the organism in infected tissues, but the shell vial culture technique coupled with rapid immunofluorescent assays enabled us to identify the virus in infected tissues in a matter of several days. Likewise, detection in the blood was expedited by this technique. Validation of less intensive diagnostics for documenting CMV pneumonia was another important advance. In the past, an open lung biopsy was the gold standard. Then bronchoalveolar lavage was found to have a sensitivity and specificity exceeding 90%, and this technique allowed transplantation clinicians to introduce a diagnostic procedure much earlier in the course of infection and to institute therapies much earlier with the hope that this would improve outcomes.

Effective therapies for CMV have also become available, such as ganciclovir, a highly potent antiviral, coupled with immune globulin, CMV immune globulin, or intravenous immunoglobin (IVIG). These therapies reduced the case fatality rates from 85% to 30% to 50%.

Truly noninvasive diagnostics have also been developed, including blood tests and assays such as the CMV pp65 antigenemia assay and a variety of PCR techniques, which can detect the virus in the blood 1 to 2 weeks before onset of disease. Finally, combining all of these advances, strategic approaches were tested and validated for prophylaxis in at-risk patients either at the time of engraftment, to be continued during the vulnerable period of time, or based on a monitoring surveillance strategy of doing tests to detect virus in the blood and then introducing the therapy at the time of virus detection.

The strategies that have been validated to be effective in reducing morbidity from CMV infection can be divided into 2 kinds based on the serologic status of the patient prior to transplantation. In patients who are seronegative, attention should be directed to trying to prevent the patient from becoming infected. A series of randomized trials conducted many years ago demonstrated that immunoprophylactic strategies were beneficial; that is, the use of IVIG or CMV immune globulin could in some cases prevent infection and, more importantly, prevent disease even when infection was not prevented. Similarly, when several donors are available to choose from, an option that occurs only in a minority of situations, it is quite clear that selection of a seronegative donor coupled with a seronegative recipient can reduce acquisition of the virus. But that precaution is effective only if CMV-negative blood products are provided, because the virus can be transmitted not only from the bone marrow or blood graft but also through blood products. In emergency situations in which the blood bank is overloaded with requests or a CMV-negative blood product is not available, the use of high-efficiency leukocyte filters can also reduce transmission of the cell-associated virus. These strategies have been validated in randomized trials.

In seropositive patients, the virus is endogenous, so immunoprophylactic strategies are not particularly useful and antiviral approaches are clearly the way to go. Both acyclovir and ganciclovir have been proven in randomized trials to be effective in reducing morbidity and, in certain cases, survival. Acyclovir is a substantially less potent anti-CMV drug because the CMV virus, in contrast to herpes simplex and varicella zoster, does not encode for a viral-specified thymidine kinase, and only small levels of the drug are phosphorylated to the active metabolite. Although acyclovir is efficacious, its potency is substantially less than ganciclovir. So most of the attention has been in evaluation of ganciclovir. Data from Atkinson et al. [2] demonstrated the rates of pneumonitis in their transplantation center before the introduction of ganciclovir into their practice and then after (Table 3). For all types of interstitial pneumonia, there was an initial incidence rate of 19%, and it decreased to 12%. When one looked at the etiologies there, it was clear that, as one

Table 2. Advances Made to Quell the Threat of CMV

- Epidemiologic studies to identify risk factors
- Improved culture technology (shell vial/IF)
- Validation of less invasive diagnostics for pneumonia
  - Bronchoalveolar lavage
- Introduction of effective therapy
  - Ganciclovir + immune globulin
- Development of noninvasive rapid diagnostics to detect active infection early
  - CMV antigen (pp65) test, PCR
- Testing of prophylaxis and preemptive strategies

Table 3. Impact of Ganciclovir on Interstitial Pneumonitis*

Pneumonitis Value	Before Ganciclovir (n = 280)	After Ganciclovir (n = 176)	P
All types	19.6%	12.5%	.03
Pneumocystis carinii pneumonia	2.9%	0.6%	NS
Idiopathic	6.3%	3.2%	NS
CMV	12.9%	1.7%	<.0005

*Data from [2].

would expect, most of this benefit was attributable to a reduction in the rates of CMV from 12.9% to 1.7%.

Preemptive versus Prophylactic Strategies

Several questions remain as to management dilemmas. The first question is whether to use a preemptive or prophylactic strategy. Both prophylactic and preemptive strategies are highly efficacious. There are some differences, and Table 4 summarizes data from various randomized studies. Prophylaxis, that is, initiation of ganciclovir at the time of engraftment and continued administration during the vulnerable period, is probably more effective in preventing all-cause CMV disease because certain breakthrough infections can occur simultaneously with the onset of the detection of the virus in the blood, which would negate the effectiveness of the preemptive therapy. Preemptive therapy refers to patient monitoring beginning at the time of engraftment continuing through the vulnerable period, with therapy initiated only in those individuals who demonstrate evidence for active infection, typically by presence of virus in blood samples. Clearly the edge is for prophylaxis with respect to achieving maximal CMV disease control. However, because that strategy treats all patients including some that never will benefit, there is an advantage to the preemptive strategy with respect to fewer toxicity episodes, particularly those associated with myelosuppression, hospitalization for neutropenic fever, bacteremia, and sepsis, and certainly it is less costly because there is a shorter period of treatment time and fewer patients who actually receive the drug.

Annual survey results indicate that most clinicians are using preemptive strategies, some are using prophylaxis, and some are using a hybrid approach in which prophylaxis is used for patients at particularly high risk and preemptive therapy in other patients. This approach, in my view, is probably the smarter thing to do. There are certain subgroups of patients that are highly vulnerable to severe morbidity and mortality, for which the preemptive strategy would entail a substantial breakthrough failure rate, including individuals at high risk for severe GVHD; recipients of T-cell depletion, either ex vivo or in vivo; and perhaps patients in the peritransplantation period who are being treated with the purine analogs such as fludarabine or cladribine and those receiving alemtuzemab. These latter points are less well developed in clinical trial data, but increasingly, even in the nontransplantation patients who are getting these agents, reports of very high, sobering rates of severe CMV and fungal infections are beginning to appear in the literature, so this is something to be on the lookout for.

Table 4. Ganciclovir for the Prevention of CMV Disease: Prophylaxis versus Preemptive Therapy

Outcome	Prophylaxis	Preemptive Therapy
CMV disease control	✓	
Toxicity		✓
Cost		✓

Choosing an Antiviral Agent

A second question is how to decide what antiviral agent to use (Table 5). Several are now available. Ganciclovir certainly is the gold standard, but it is associated with myelosuppression. Foscarnet is an alternative. It has a different mechanism of action, and as is addressed in the following article by Dr. Boeckh, it could be useful in isolates that may be resistant. Unfortunately, it is less well studied and although not myelosuppressive, it does have a different set of toxicities, particularly nephrotoxicity. Cidofovir is a very interesting agent, not only for this virus but certain other viruses as well. But it has not been well studied in this population. There are substantial toxicities to be aware of in terms of nephrotoxicity and myelosuppression, so patients receiving cidofovir must be monitored very carefully. Oral ganciclovir eliminates the need for parenteral therapies, which most CMV antivirals necessitate. Drawbacks of oral ganciclovir are poor bioavailability by itself and some resistance in patients with HIV infection who have received ganciclovir orally for long-term therapy. In these patients the emergence of drug resistance appeared to be greater than in patients getting IV therapy, because the levels are not entirely suppressive. Finally, the antiviral valganciclovir has substantially better bioavailability, a characteristic that will be addressed in a subsequent article.

Treatment Duration

A third question is how long patients should receive treatment for CMV infection. The initial studies were done up until day 100, 110, and 120, but clearly a variety of studies show that shorter periods of therapy are effective. The best study is a randomized trial by John Zaia et al., City of Hope [3] (Table 6). Looking at rates in CMV disease, episodes of bacterial sepsis, and survival, they found that a shorter treatment period, in this study 6 weeks compared to 12 weeks, showed no reduction in the ability to control the CMV. There were fewer episodes of sepsis, particularly beyond 6 months, and in fact there appeared to be improved survival with the shorter treatment period. Several other groups have used even shorter periods of time, 3 weeks, with certain other dose schedules. These shorter episodes of therapy are effective, but if therapy is stopped then patient monitoring must be resumed because patients remain vulnerable to recurrences and may require additional therapies.

Late-Onset Disease

A fourth issue is late-onset CMV disease, disease occurring beyond 100 days posttransplantation. Late-onset CMV disease, formerly an infrequent problem, is becoming more prevalent, as indicated by data from Seattle obtained between 1986 and 1994. Figure 2

Table 5. Choosing an Antiviral Agent

• IV ganciclovir	• "Gold standard" • Myelotoxic
• Foscarnet	• Less well studied • Nephrotoxic
• Cidofovir	• Not well studied • Nephrotoxic
• Oral ganciclovir	• Poor bioavailability (6%-9%) • Resistance (6.5% in HIV patients)
• Valganciclovir	• Much improved bioavailability (60%)

Table 6. CMV Therapy: How Long Should Patients Be Treated?

Parameter	12 wk	6 wk	*P*
CMV disease			
<6 mo	11%	7%	.99
>6 mo	35%	22%	.40
Sepsis			
<6 mo	54%	32%	.49
>6 mo	64%	27%	.03
Survival at 1 y	50%	63%	.04

shows that the rates of CMV disease prior to day 100 declined dramatically from 32% down to 6% with the use of strategies to control CMV. There was a slow but relentless climb, however, in the incidence of late-onset CMV disease, going from about 3% up to about 16%. Clearly this increase indicates that late-onset CMV disease poses a major challenge. Each of the two strategies, preemptive and prophylactic, appear to have a slightly different impact on the risks for late CMV infection. Data from Michael Boeckh et al. (Table 7) [5] showed that with prophylaxis the rate of disease prior to day 100, 2.7%, was lower than with preemptive therapy, for which it was 14%. Beyond day 100, however, rates for prophylaxis and preemptive therapy were 13% and 6%, respectively, so there was a difference. The overall protective rates were similar, but the time of onset was quite different with the two strategies.

The risk factors for late-onset CMV disease are beginning to be identified. Viral factors include the use of ganciclovir and high viral burden early after the transplantation. Host immune factors have also been found to be important, particularly chronic GVHD, treatment with alternate donor stem cells, low CD4 counts, and the absence of early protective anti-CMV antibodies and cytotoxic responses.

Conclusion

So in conclusion, it is quite clear that although this infectious pathogen represents a serious threat to our transplant recipients, the introduction of noninvasive diagnostics, active therapeutics, and the testing of effective therapies have dramatically changed how we manage CMV patients and have improved disease outcome, particularly for early CMV morbidity and mortality. However, these advances have been traded for and slightly offset by the increase in rates of late-onset infection and disease, to which chronic GVHD and impaired immune responses are major contributors. In my view, evaluation of late prevention strategies is a high priority in our patients.

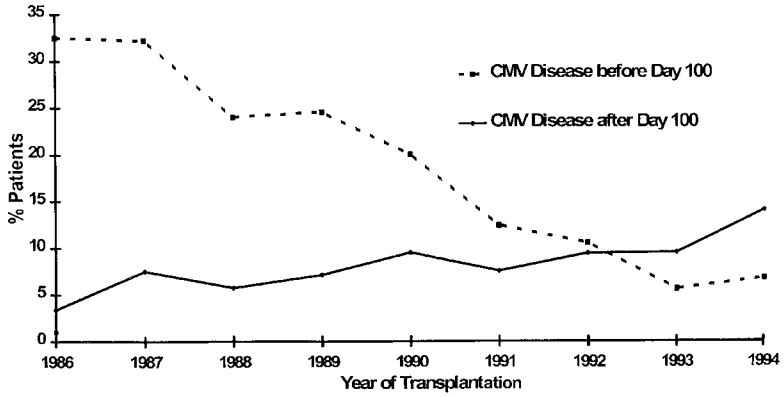

Figure 2. Late-onset CMV disease. Data show that treatment strategies resulted in a decrease in early CMV disease, but late CMV disease continued to increase [4].

Table 7. Effect of Ganciclovir Strategy on Late CMV Disease*

CMV Disease	Prophylaxis	Preemptive	*P*
Up to day 100	2.7%	14.1%	.002
Beyond day 100	13.4%	6.1%	.2
Total before day 400	16.1%	20.2%	.42

*Data from [5].

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CMV Diagnosis and Resistance

Michael Boeckh, MD

CMV Diagnostics

There are now a number of acceptable diagnostic tests for cytomegalovirus (CMV), basically antigenemia-based techniques and polymerase chain reaction (PCR)-based techniques. Certain criteria and patient characteristics can be applied in selecting which test to use among the number of options now available. Sensitivity, specificity, and predictive values for infection and disease are important considerations. Although PCR is a very powerful technology, in practice the performance of PCR assays can be quite variable. Because the CMV virus replicates much faster in patients who have severe immunosuppression, sensitivity of the assay is important in these extremely high-risk patients, but the edge that you get through an ultrasensitive assay becomes apparent only in such patients. In moderate-risk patients such as matched-related transplant recipients, the assays that are usually used such as antigenemia or qualitative PCR are quite good, and the studies support the effectiveness of these strategies. If quantitative assays are used, and there is a trend toward their use, assay variability is an important factor, but it is often overlooked in clinical practice. All assay techniques have

limitations in terms of assay variability, so an important question in analyzing assay results is what values indicate a true increase in viral load. If one PCR level shows 1100 copies and the next week shows 1200 copies, this is really not a true increase.

The gold standard for a diagnostic test, of course, is performance in the clinical setting, and optimally you would like to have one prospective randomized trial for each available assay versus the gold standard. Of course that is not going to happen, so the second best option is to evaluate the assay in a time-to–first detection fashion, which provides a pretty good sense of what the assay can deliver, and then look at the outcome results for preemptive therapy strategies that are based on those assays.

A few key points are important in evaluating options with diagnostic techniques:

- Assay sensitivity is important in highly immunosuppressed patients because of the rapid increase in viral load.
- PCR assays can be made extremely sensitive, and in that context primer selection, sample amount, and DNA extraction methods are important determinants.
- PCR assays can be optimized for plasma, leading to sensitivity similar to that of cellular assays.

In our own lab, we did two things to optimize our plasma-based PCR assays, which actually used to be sensitive only at a level of 500 copies per mL, what most of the plasma-based assays were able to achieve. We used a double primer in the UL55 and the UL123 region and we used an automated extraction method (Table 1). When this method was evaluated in a time-to-event fashion in which 23 patients weekly were sampled, plasma was found to perform quite well, and a method using plasma has the obvious advantage of being very simple. There is another study, recently published in the *Journal of Clinical Microbiology*, in which the Cobas quantitative CMV assay was modified by adding centrifugation steps [1]. These technologies have been used for assays for human immunodeficiency virus (HIV) and hepatitis C. The sensitivity of the assay could be increased from about 500 copies per mL to 20 to 50 copies per mL. Table 2 presents data on how this assay performed in the clinical setting. Basically, the amount of CMV detection increased by 50%.

Table 2. Improved Plasma PCR: Modified Roche Cobas Amplicor Assay*

	No. (%) of CMV-Positive Samples	
	All Samples (n = 319)	Subgroup with Comparison of 3 Assays (n = 214)
Ultrasensitive Amplicor CMV monitor test	84 (26)†	56 (26)†
pp65 antigen assay	38 (12)†	28 (13)
Standard Amplicor CMV monitor test		25 (12)†

*Data from [1].
†$P < .01$.

Drug Resistance

Ganciclovir is still the drug used to treat CMV disease in the majority of cases, so most of the available reports on drug resistance actually address ganciclovir resistance. Drug resistance can occur with any of the available agents, however. The most common are

- Ganciclovir
- Oral ganciclovir
- Valganciclovir
- Foscarnet
- Cidofovir

One thing that is apparent in the literature is that there are many more reports from solid organ transplantation patients than from stem cell transplantation patients (Table 3) [2-9]. The drug resistance incidence figures that have been reported for stem cell transplantation patients range between 0% and 3.8%. In the solid organ transplantation setting, almost all the resistance seems to be restricted to the donor-positive recipient-negative (D+/R–) constellation. There is virtually no resistance in the R+ patients, with the exception of lung transplant recipients [3]. For stem cell transplant recipients, there is no information on the impact of the source status. Table 4 lists data that are in the literature so far [6-11], according to the setting in which the investigators looked at the issue of resistance. One study done in Seattle almost 10 years ago looked at 12 patients with CMV pneumonia and found only 1 case of ganciclovir resistance that was also associated with therapy failure [10]. Overall it was concluded that ganciclovir resistance is not a major reason for therapy failure. There are several papers that looked at the setting of documented CMV asymptomatic infection detected either by culture or, in the most recent data, by antigenemia assay. Results show a range of either no detection, in the most recent study by Dr. Bolvin from Canada (Gilbert et al. [6]), or up to 6.7% in the setting of rising antigenemia in 1 of 15 patients [7], but generally detection was low. There were 2 studies [8,9] of highly immunosuppressed recipients of unrelated or haploidentical transplants. In these studies the incidence of ganciclovir resistance ranged between 4% and 8%. The most recent report from Israel [9] actually found 2 cases among 26 patients, which amounts to almost 8% in a cohort of haploidentical patients. These results indicate that these are the types of patients for which we have to be aware of the risk of ganciclovir resistance.

Resistance Factors

What are the factors that indicate the presence of or risk for resistance? It is clear from the literature at this point that no single

Table 1. PCR for CMV DNA: Is Plasma Adequate?

- Method: real-time double-primer PCR (UL55/UL123)
- Weekly longitudinal sampling (N = 23 stem cell transplant recipients)
- Results:
  - —Plasma = peripheral blood lymphocytes 26%
  - —Plasma before peripheral blood lymphocytes 60%
  - —Plasma after peripheral blood lymphocytes 14%

Table 3. Ganciclovir Resistance Incidence in Transplantation Patients*

Organ	All	Recipient+	Donor+/Recipient–
Kidney	0.5%-1%	0%	2.6%-5%
Liver	0%-0.25%	0%	0%-3%
Heart	0.3%	0%	1.4%
Kidney-pancreas	13%	0%	21%
Lung	2.2%-9%	0%-4.4%	10%-27%
Stem cell	0%-3.8%	N/A	N/A

*Data from [2-9]. N/A indicates not analyzed.

Table 4. Ganciclovir Resistance Incidence in Hematopoietic Stem Cell Transplantation Patients

Reference	Setting	N	Incidence
CMV disease			
Slavin 1993 [10]	CMV-IP	12	8.3%
CMV infection			
Erice 1998 [11]	CMV infection	15	6.7%
Nichols 2002 [7]	pp65 antigen	119	0.8%
	Rising pp65 antigen	15	6.7%
Boivin 2001 [6]	pp65 antigen	34	0%
All Patients			
Eckle 2000 [8]	Unrelated donor/haploidentical	79	3.8%
Wolf 2003 [9]	Allogeneic/haploidentical	138	1.4%
	Haploidentical only	26	7.6%

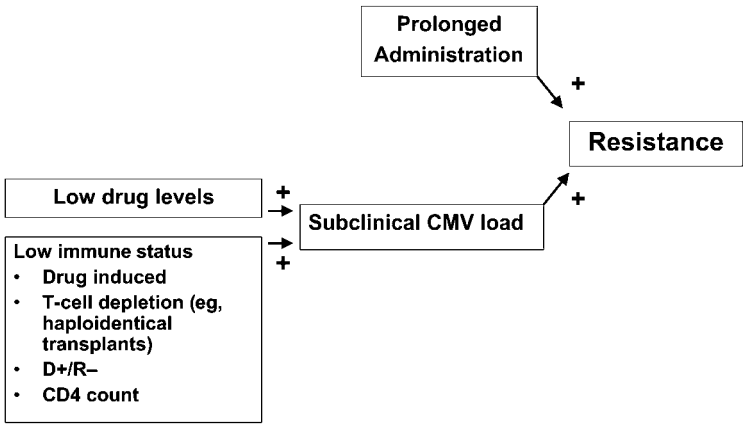

Figure 1. Factors contributing to resistance against anti-CMV drugs.

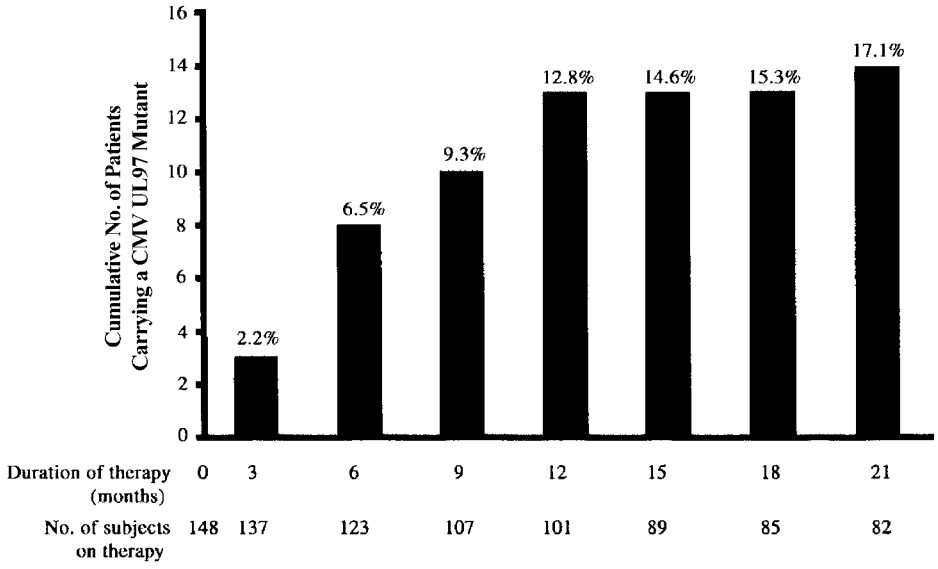

Figure 2. Impact of duration of treatment on drug resistance in patients treated with valganciclovir for CMV retinitis.

factor is responsible (Figure 1). Data from the solid organ setting indicate that prolonged drug administration has to occur before resistance to ganciclovir or any other agent is suspected. From a pathogenesis point of view, there probably also has to be subclinical CMV reactivation in the presence of prolonged drug administration. The factors that favor this subclinical reactivation are low immune status, which could be drug-induced; T-cell depletion, which could occur in the solid organ transplantation setting or the D+/R– setting; or low CD4 count. Only if these factors come together is antiviral-drug resistance likely.

Figure 2 shows data about the impact of duration of administration on resistance [12]. This data on patients treated with valganciclovir comes from the HIV setting. During early months of the therapy there was virtually no resistance, but resistance increased after several months. These results are relevant to the stem cell transplantation setting because we are now actually moving toward the use of more drug for a prolonged period as a prevention strategy for late CMV disease. The stem cell transplantation setting, however, differs somewhat from the solid organ transplantation setting. In the stem cell transplantation setting, at least for traditional myeloablative transplantation, CMV-specific immune reconstitution is an important issue. For that to occur, the host actually has to be exposed to the antigen, at least to some extent, so the balance has to be between brief antigen exposure, which can stimulate immune reconstitution, versus prolonged subclinical reactivation in the presence of drug, which can cause resistance (Figure 3). So duration of CMV reactivation makes a difference. If CMV could be suppressed 100% with a hypothetical drug, resistance would probably not be a major problem, but CMV-specific immune reconstitution would probably be delayed.

Detection of Resistance

Table 5 summarizes methods for detection of resistance to ganciclovir or any drug. The conventional methods are cumbersome and really not useful for clinical practice. These methods, which usually require an isolate, have been used for some of the studies mentioned here. The future of resistance detection is clearly molecular testing, methods for which have been published. We know exactly where the mutations are that go along with resistance development for ganciclovir, foscarnet, and cid-

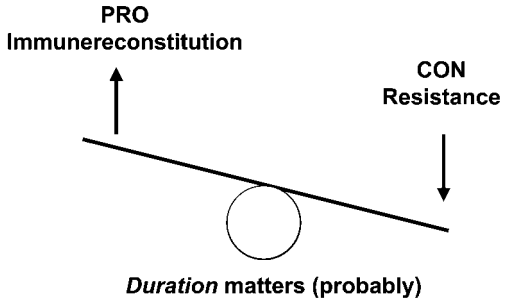

Figure 3. Subclinical infection: the right balance. For CMV-specific immune reconstitution to occur in the stem cell transplantation setting, the host has to be exposed to the antigen, at least to some extent. A prolonged subclinical reactivation in the presence of drug could lead to resistance, but 100% CMV suppression, if it could be obtained, would probably delay CMV-specific immune reconstitution.

ofovir, so direct sequencing from DNA samples will be the way to go in the future.

A method already in use is detection of increases in CMV viral load in patients undergoing treatment for CMV infection. What is a true increase of viral load? If antigenemia or PCR assay results for a patient on ganciclovir show that the viral load level has gone up, should treatment be switched automatically to an alternative drug like foscarnet or cidofovir? Assay variability, a mentioned previously, is very important in this context. Quantitative DNA assays generally have a lower assay variability than antigenemia assays. For most DNA-based assays, the coefficient of variation is less than 0.3, so viral load increases of more than half a log are likely to indicate a true increase. This cut-off value has not really been evaluated in comparative studies, but one can extract these data from the literature.

Are viral load increases on antiviral therapy indicative or predictive for resistance? The answer depends on the situation. In the low-risk situation, if a ganciclovir-naive patient has an increase of viral load during the first 2 to 3 weeks of therapy and the patient is in a low-risk transplantation setting such as stem cell transplantation with no in vivo or ex vivo T-cell depletion, the patient is not on prophylactic hydrosteroids, or the patient is a kidney, liver, or heart transplantation patient in the R+ or D+/R– setting, resistance is extremely uncommon for 95% of all such patients. This scenario, which is outlined in Figure 4A, is one we have actually observed (Figure 5). Dr. Nichols et al. have described some of these patients who had antigenemia increases that were found to be mainly due to the underlying steroid dose [7]. A moderate level of resistance was found in only one patient, and that patient eventually responded to ganciclovir and some foscarnet, so in this particular clinical situation ganciclovir resistance is extremely rare.

There is, however, a high-risk scenario (Figure 4B) occurring in a ganciclovir-experienced patient who has either received prophylactic preemptive therapy or, as is increasingly common, pretransplantation therapy and has an increase of viral load for more than 2 weeks. This type of patient has to be in a high-risk transplantation setting, which is lung, kidney, or pancreas transplantation or,

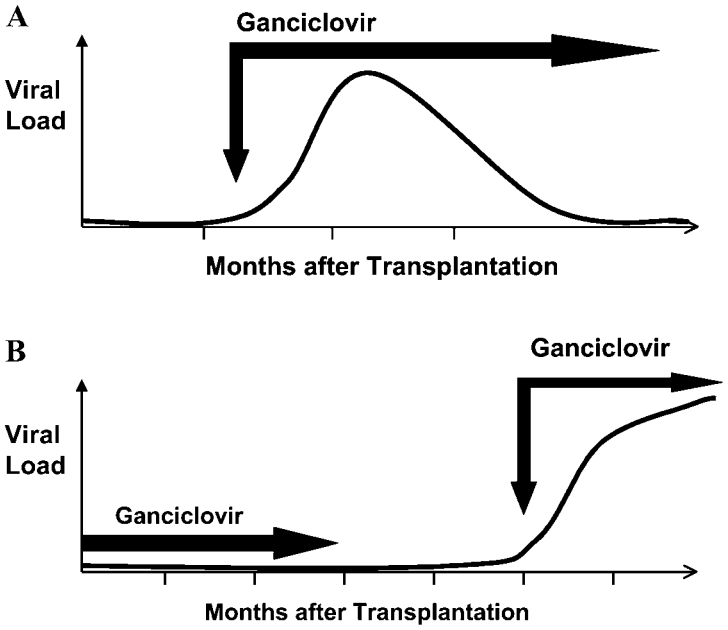

Figure 4. A, First scenario: low-risk situation. Ganciclovir-naive patients in the transplantation setting who show an increased viral load during the first 2 to 3 weeks of ganciclovir therapy are at low risk for resistance if they received a hematopoietic stem cell transplant without in vivo or ex vivo T-cell delpetion or if they received kidney, liver, or heart transplants in the R+ or D+/R– setting. B, Second scenario: high-risk situation. Ganciclovir-experienced patients (who have previously received ganciclovir for prophylaxis, preemptive therapy, or pretransplantation use) who show an increased viral load for more than 2 weeks after beginning ganciclovir therapy are at high risk for resistance if they are in a high-risk transplantation setting such as D+/R– lung, kidney, or pancreas transplantation or hematopoietic stem cell transplantation with severe T-cell depletion or immunosuppression (eg, haploidentical transplantation).

Table 5. Drug Resistance against CMV: Methods of Detection

- Conventional methods: cumbersome
  - —Isolate required
  - —Examples: plaque reduction, Hybrivix, simian virus, and flow cytometry–based methods
- Molecular methods: the future
  - —Isolate or DNA
  - —PCR of mutation "hot spots"
  - —Direct sequencing
- Viral load increases: what we have today

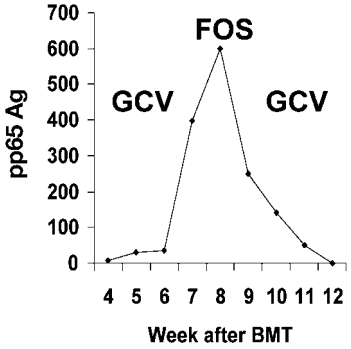

Figure 5. pp65 increases in ganciclovir (GCV)-naive HSCT recipients: rarely caused by antiviral resistance. Analysis results from 21 isolates from 15 patients showed that 6 of 6 pretherapy isolates were sensitive. After a mean GCV treatment duration of 4.5 weeks (range, 1-10 weeks), 14 of 15 isolates were sensitive. One patient with GCV resistance responded to GCV and foscarnet (FOS).

in our situation, stem cell transplantation with severe T-cell depletion or immunosuppression, eg, haploidentical transplantation.

Summary

Ganciclovir resistance remains rare but occurs in specific clinical settings such as those outlined in Figure 4. Resistance is generally not seen during the first preemptive therapy course in ganciclovir-naive patients. CMV viral load increases in drug-naive subjects, so during early treatment such an increase constitutes a low-risk situation and resistance is generally unlikely. However, after previous significant exposure to any antiviral drug and in a high-risk setting, the possibility of resistance should be considered, and potentially a drug change will be necessary.

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Emerging Treatment Options for CMV in Stem Cell Transplantation

Garrett Nichols, MD

Introduction

Late cytomegalovirus (CMV) disease is a primary emerging issue in stem cell transplantation with regard to CMV. The question of who is at risk for CMV disease late after stem cell transplantation and some issues about how these patients should be managed are addressed here. New and emerging therapeutic options are also discussed, such as the orally bioavailable drugs valganciclovir and the investigational agent 1263W94 (Maribavir), immunotherapy such as passive T-cell transfer, and the potential for CMV vaccines in the prevention of CMV disease for these at-risk individuals.

Late CMV Disease

Late CMV disease is currently the primary problem in our stem cell transplantation patient population. With the use of preemptive and prophylactic ganciclovir strategies, there has been a dramatic decrease in the incidence of early CMV disease during the first 100 days posttransplantation but a corresponding increase in the incidence of late CMV disease. This increase clearly makes late CMV disease a complication that more and more patients suffer and clinicians have to deal with. Dr. Boeckh and colleagues recently published a report in ***Blood*** of a large cohort study looking at the epidemiology of late CMV disease after stem cell transplantation [1]. In this cohort of 146 patients (Table 1), the incidence among CMV seropositive individuals was approximately 18%, with a median day of onset of 170 days after transplantation.

Some of the manifestations of CMV disease are common, and some of these such as CMV pneumonia and CMV gastrointestinal disease are very well described in the stem cell transplantation setting. Incidence rates are also increasing of unusual manifestations for the stem cell transplant recipient such as CMV retinitis or CMV encephalitis, as has been reported in [1] and in the report by Dr. Wolf et al. in Jerusalem [2] that was previously referenced in Dr. Boeckh's article.

We evaluated the risk factors for CMV disease in two ways. First we looked at individuals who were being evaluated for discharge from the transplantation center, which usually occurs between day 80 and 100 after transplantation. When just the factors that we knew of at the time of discharge were looked at, several factors dropped out as significant predictors for late CMV disease. These factors

Table 1. Late CMV Disease after SCT Epidemiology*

• Incidence (CMV seropositive)	26/146 (17.8%)
• Day of onset	169 (96-784)
• Risk factors at discharge evaluation	
—Early CMV reactivation	
—GVHD (acute or chronic)	
—Lymphopenia	
—Lack of CMV lymphoproliferative response at day 80	

*Data from [1].

Table 2. Late CMV Disease after SCT: Predictors with Late Virologic Monitoring*

Covariate	RR (95% CI)	Adjusted RR (95% CI)
Lymphocytes ≤300	7.2 (3.1-17.0)	9.4 (3.8-23.5)
pp65 antigenemia (any)	4.0 (1.4-11.7)	5.3 (1.5-19.1)
>5 Cells/slide	4.0 (1.3-12.1)	6.1 (1.7-21.5)
>50 Cells/slide	5.5 (1.5-20.6)	8.7 (2.1-36.5)
Plasma PCR		
>1000 Copies/mL	2.1 (0.5-8.3)	6.2 (1.0-39.2)
>10,000 Copies/mL	3.0 (0.8-12.3)	12.3 (1.8-85.1)

*Additional factors evaluated: chronic GVHD, donor type, CD4 <50. RR indicates relative risk; CI, confidence interval.

Table 3. Late Mortality after SCT: Effects of CMV Infection in Multivariate Models

Factor	RR (95% CI)	P
CMV disease	2.3 (12.-4.2)	<.01
Lymphocytopenia	3.6 (1.9-6.7)	<.01
CMV reactivation (DNA+)*		
Any level	13.1 (1.3-132.7)	<.05
>1000 copies/mL	22.6 (2.2-227.8)	<.05

*Adjusted for lymphocytopenia, chronic GVHD, aspergillosis.

included the presence of early CMV reactivation as determined by the antigenemia assay and the presence of acute or chronic graft-versus-host disease (GVHD) at the time of evaluation. Figure 1 shows the cumulative CMV incidence curve. The incidence of CMV disease among patients with any acute or chronic GVHD was approximately 21%, whereas in individuals who did not have GVHD before day 95 the incidence was much lower, approximately 4%. Lymphocytopenia, a lymphocyte count of less than 300, at the discharge evaluation was also a significant predictor. Finally, the lack of CMV lymphoproliferative responses at day 80 was also predictive of subsequent CMV disease.

The second way that we looked at the predictors for late CMV disease was to consider not only those factors that we knew of at day 80 but also the value of ongoing virologic monitoring. Individual patients sent us blood samples, which we tested for the antigenemia assay and plasma polymerase chain reaction (PCR) for CMV DNA. These results (Table 2) were not used to guide preemptive therapy because their predictive value was not known at that particular time point. Again, lymphocytopenia sorts out in a time-dependent fashion to be an independent predictor after adjusting for other factors. The presence of any pp65 antigenemia was associated with a 5-fold increase in risk, with an increase in risk according to the level of viral load that was detected by antigenemia. When we looked at plasma PCR results showing more than 1000 copies per mL after controlling for other factors, including lymphocytopenia, chronic GVHD, donor type, and low CD4 counts, the presence of a positive plasma PCR greater than 1000 copies per mL was associated with a 6-fold increased risk in the incidence of late CMV disease, and a viral load greater than 10,000 copies was associated with a 12-fold increase in risk.

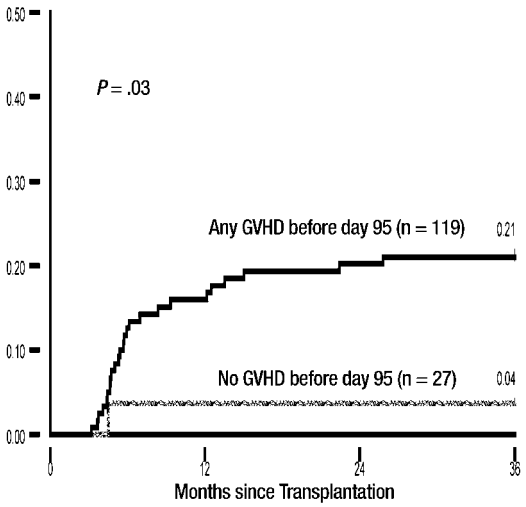

Figure 1. Late CMV disease after SCT: epidemiology. Data from [1].

Looking at the effects of CMV infection and CMV disease on late mortality after CMV transplantation (Table 3), as expected late CMV disease is predictive of mortality, with a 2-fold increase in risk for all-cause mortality late after transplantation. Lymphocytopenia is also a significant risk factor for late CMV disease, even after adjusting for chronic GVHD. However, CMV reactivation in and of itself, even after controlling for lymphocytopenia and GVHD, was associated with a significant risk for all-cause mortality. Patients who had any level of CMV reactivation as determined by the PCR assay, PCR for CMV DNA, had a 13-fold increase in risk of all-cause mortality. In patients who had reactivation at greater than 1000 copies per mL the risk was increased almost 23-fold. So we would conclude that CMV disease is common late after stem cell transplantation, that either CMV infection or CMV reactivation in addition to CMV disease predicts mortality, and thus that preventing reactivation may be important.

Prevention of Late CMV Disease

The risks for CMV disease may be identified at day 80 to 100 after transplantation. These risks include early reactivation of CMV, GVHD, and lymphocytopenia. It is obvious that new strategies are needed in this patient population. How can late CMV disease be prevented in these individuals? There are important logistical considerations for transplantation centers that frequently send their patients back home to referring physicians, many of whom do not have a lot of experience in managing transplant recipients.

Prophylactic Strategy

The ideal obviously would be a targeted prophylactic agent that could be used for patients who are at high risk. This agent

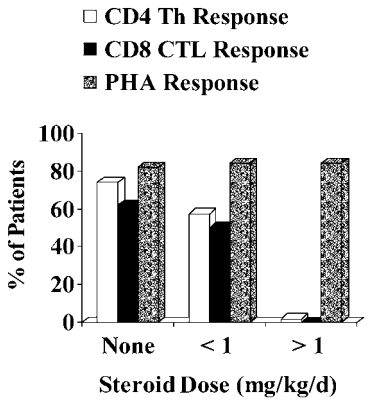

Figure 2. Impact of steroid dose on CMV-specific CD4 T-helper (Th) and CD8 cytotoxic T-lymphocyte (CTL) response. PHA indicates phytohemagglutinin.

would be very safe and thus there would be no need for safety monitoring for drug toxicity, and it would also be highly effective so that we wouldn't need to monitor the patient for virologic reactivation. Unfortunately, this ideal targeted prophylactic agent does not yet exist. Currently available agents include valganciclovir and valacyclovir. Valacyclovir is not as potent a preventive agent as acyclovir; however, it is effective in decreasing CMV reactivation, at least in the early period after transplantation. Valganciclovir is the oral form of ganciclovir and is certainly more effective in suppressing CMV. But there are tradeoffs regarding efficacy versus toxicity. Valganciclovir, for example, has the potential for causing neutropenia.

Preemptive Strategy

The alternative to prophylaxis is to continue a preemptive strategy with ongoing virologic monitoring and preemptive therapy. In the most immunosuppressed patients virologic monitoring is required on at least a weekly basis because of the rapidity with which CMV would replicate in these individuals. Coordination of such monitoring is difficult in a late setting outside of the transplantation center and involves educating the referring physician and emphasizing the importance of the preemptive strategy in order to prevent CMV. Coordination and compliance are significant issues in the long-term follow-up setting. However, given that the toxicity and the efficacy of these prophylactic strategies are unknown, most centers use a preemptive approach, for which an issue is when the preemptive approach should be started and when it can be stopped. For patients who are not involved in our randomized control trial, we have used a preemptive approach that includes weekly monitoring for at-risk patients until day 365 after transplantation. Patients who are deemed at risk are those who have early reactivation of CMV disease or receive steroids for chronic GVHD. We test these individuals every week and if we detect CMV reactivation we give them preemptive ganciclovir therapy for 2 or 3 weeks and then resume preemptive monitoring. Weekly testing, however, is problematic for many patients, particularly those who are otherwise doing well and have no other reason to get their blood drawn at such frequent intervals. Testing intervals can be increased to every 2 weeks in patients who are not significantly immunosuppressed, who are receiving steroids at a tapering dose, and whose current steroid dose is less than 1 mg/kg. What is the significance of steroid dose? A study from our group that was recently presented [3] showed that the corticosteroid dose has a large impact on the ability of the individual to mount a CD4 T-helper response and a CD8 T-helper response (Figure 2). These responses were detected in up to 80% of patients who had not received corticosteroids, and they were only slightly decreased in patients who were receiving a current steroid dose of less than 1 mg/kg. In patients who were receiving corticosteroid doses of greater than 1 mg/kg, however, these responses were virtually undetectable. Therefore CMV immunity, which we know is important in controlling this virus, is significant, and patients who are receiving a steroid taper with a current dose of less than 1 mg/kg and have not reactivated in the previous month can go to every-other-week testing.

The second question is which agent do we use for preemptive therapy in this particular situation? Valganciclovir represents a potentially significant advance in therapy for these patients, especially those who have already had their catheters discontinued and don't therefore have intravenous (IV) access for IV ganciclovir. Ganciclovir has been our antiviral of choice for treating CMV after stem cell transplantation, but its oral bioavailability is extremely low (3%-9%) and IV administration adds cost and inconvenience. We know that valganciclovir is well absorbed orally, at least in the human immunodeficiency virus (HIV) and liver transplantation settings. Liver transplant recipient data in Figure 3 show pharmacokinetic (PK) curves indicating the overall area under the curve and the peak, the maximum concentration, are comparable

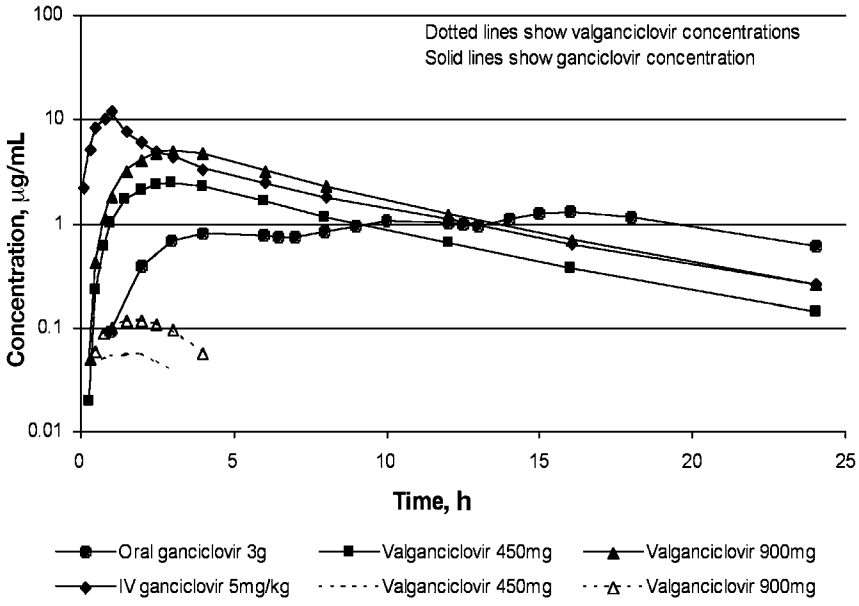

Figure 3. PK curves for ganciclovir and valganciclovir in liver transplant recipients. Overall area under the curve and maximum concentration are comparable between 5 mg/kg of ganciclovir given IV and 900 mg of valganciclovir given orally.

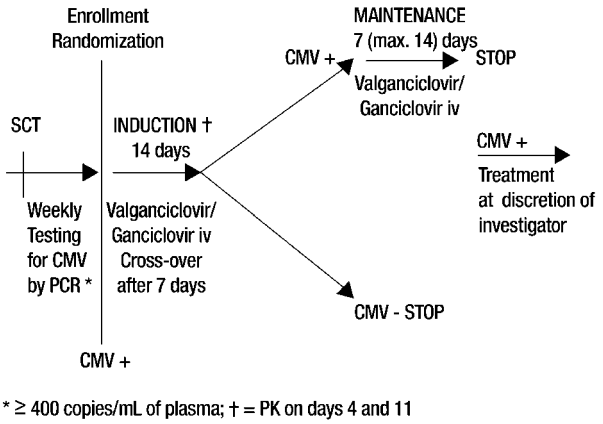

Figure 4. Valganciclovir for PCR-based preemptive therapy of CMV infection after allogeneic SCT: a multicenter German-Swiss study.

between 5 mg/kg of ganciclovir given IV and 900 mg of valganciclovir given orally.

What are some of the potential applications of these agents in the late period after stem cell transplantation? This past year, an article in the *New England Journal of Medicine* summarized late complications of stem cell transplantation [4]. In recommendations for treatment of reactivation of CMV infection late after allogeneic or autologous hematopoietic stem cell transplantation, valganciclovir was listed as the agent of choice. We don't yet know for certain, however, that this treatment is entirely safe in this particular patient population. Efficacy requires absorption, and PK in solid organ transplantation and HIV patients may not exactly approximate those in stem cell transplant recipients. The issues in stem cell transplant recipients that are unique are that these patients may have impaired gut integrity so that early after transplantation mucositis may interfere with absorption, and late after transplantation the presence of gut GVHD is also complicating. We know that absorption is substantially increased if patients eat a good meal. The PK studies shown in Figure 3 involved patients who ended up eating a prescribed breakfast that included eggs, milk, cereal, toast, orange juice, coffee, etc. If you know stem cell transplantation patients who eat that way every day then I would like to meet them. It is important to recognize that absorption is increased significantly with food, so we may not have the same confidence that optimum absorption is occurring if the individual is not eating well. Finally, as with ganciclovir, neutropenic toxicity is a potential issue with valganciclovir.

The other issue that we have to consider is interpreting quantitative virologic response and looking at viral load in patients who are being monitored with ongoing virologic therapy. We know that approximately one third of patients will have an antigenemia increase without resistance because of their underlying corticosteroid dose. As has already been shown, this is not an assay-specific phenomenon that occurs only with the pp65 antigenemia assay, it also occurs when patient CMV DNA is analyzed by PCR. Rising viral load was not associated with resistance in this study; it was associated with a degree of immunosuppression as reflected in the current corticosteroid dose. So the antiviral dose is key. It is important to continue induction if the patient has increasing viral loads, but if oral valganciclovir is used in induction therapy for initial reactivation, we have to be concerned not only about what the patient's current immunosuppressive level is but also about whether the patient is actually absorbing the drug. So until PK and randomized control trial data on absorption are available, IV induction may indeed be preferred in this particular setting; data from ongoing studies, however, indicate that valganciclovir may be effective for maintenance therapy.

Figure 4 shows data from a multicenter German/Swiss study that is looking at valganciclovir versus IV ganciclovir for early preemptive CMV therapy within the first 100 days after transplantation. Patients are randomized at the time of reactivation to receive either IV ganciclovir or oral valganciclovir. Patients get induction for 14 days and then they go to maintenance therapy. This study, which includes PK monitoring on days 4 and 11, seeks to answer the important question: "Does mucositis impact the effectiveness of oral valganciclovir early, and does mucositis affect the pharmacokinetics of valganciclovir in our patients?"

We are conducting a multicenter United States study to look at the issues surrounding late CMV prevention (Figure 5). This study is a randomized double-blind placebo-controlled trial of valganciclovir in which patients are evaluated for eligibility at day 80 to 120 after transplantation, are then randomized to oral valganciclovir 900 mg once a day versus placebo, and then continue to receive their assigned drug and are followed until day 270 after transplantation. The rationale for use of the oral form in this particular setting is that it is ideal for long-term prophylaxis and follow-up, which would be difficult to accomplish with IV ganciclovir. As discussed earlier, CMV infection is associated with mortality in excess of CMV disease, and thus the prespecified goal in this particular study is to prevent CMV reactivation in

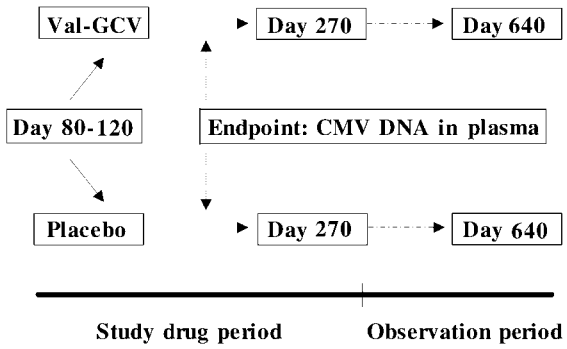

Figure 5. Late CMV prevention trial: a multicenter United States study.

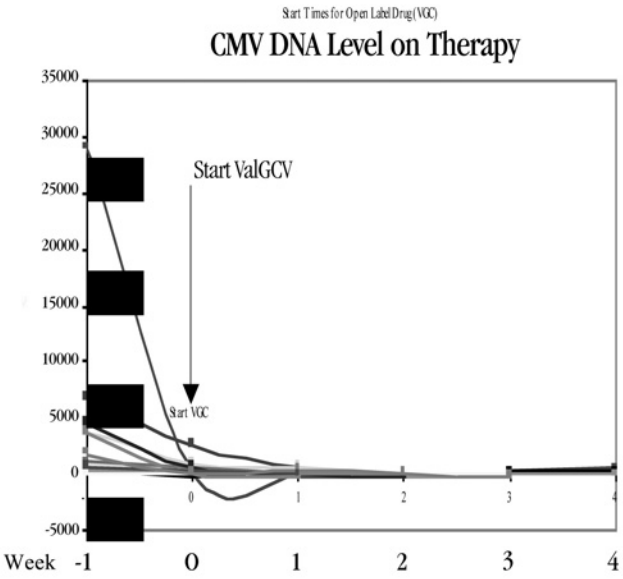

Figure 6. Valganciclovir for late CMV prevention: efficacy during open-label therapy.

order to prevent some of these hypothesized indirect effects of CMV after stem cell transplantation. All of the patients participating in this study are tested weekly for CMV DNA by quantitative PCR and plasma, and patients with significant reactivation, greater than 1000 copies per mL, receive preemptive therapy in the form of IV ganciclovir induction for 1 week. If their viral load decreases, then they receive follow-up oral maintenance with open-label valganciclovir. So this study seeks to determine whether oral valganciclovir as a prophylactic measure is 100% effective. If so, monitoring would not be required for patients receiving this treatment, a result that would lead to huge savings in cost and also complication for these patients, who currently must have their viral load checked on a weekly basis. If this treatment is effective in preventing CMV reactivation, then it must be determined how much neutropenic toxicity is associated with valganciclovir in the late setting and what will be the consequences in terms of bacterial and fungal infections in the late setting. The outcome of this study depends on which issue predominates: the abrogation of CMV-induced indirect effects, the immunosuppressive effects of CMV per se, or the issue of valganciclovir-induced neutropenia.

Figure 6 presents preliminary data from our study on the efficacy of valganciclovir for preemptive therapy in this particular patient population. The study is still blinded so we don't know what the issues are in terms of valganciclovir's effectiveness in reactivation issues. Once patients suffer CMV reactivation, they get IV ganciclovir followed by oral valganciclovir open label. We have had a total of 13 episodes of reactivation thus far in 8 patients on this study. These patients were treated with IV ganciclovir, usually for 1 week, and then started valganciclovir thereafter. The virus cleared with preemptive therapy in all the patients. In other words, there was no significant rebound after the patients had decreasing viral loads with 1 week of IV ganciclovir. The median time for clearance was 4 weeks, with a range of 3 to 6 weeks. In patients whose CMV reactivated, there were no cases of CMV disease when these patients continued on oral valganciclovir for maintenance.

Other options for treating CMV infection are becoming available. 1263W94, also called Maribavir, is a drug of the class of the benzimidazoles, which have been investigated. These drugs are ribosides with a unique mechanism of action that is distinct from the mechanism of action of all the other CMV agents that have been tested to date. This drug is active against both laboratory and clinical strains, including those that are ganciclovir resistant [5]. In an HIV patient model that looked at semen CMV titers, there was mean 3 to 4 log reduction [6]. The agent is extremely bioavailable when administered orally and has linear PK, but it is metabolized by the hepatic CYP3A4 metabolism mechanism so drug-drug interactions are a concern. 1263W94 is safe and not genotoxic. It does not cause myelosuppression or renal toxicity. The most common side effects are taste disturbance, rash, and diarrhea. This drug is a promising agent for future study in stem cell transplantation recipients.

Immunotherapy also has potential for the future. The passive transfer of CMV-specific CD4 and CD8 clones is possible, and CMV DNA and conjugated peptide vaccines are also in development.

Conclusions

Late CMV disease remains problematic after stem cell transplantation. Patients who are at risk are those who are CMV seropositive with early reactivation and are receiving steroids for GVHD. There are new therapeutic options that are emerging, including valganciclovir and Maribavir, both of which appear promising, and we look forward to a future in which immunotherapy may be used to treat these patients.

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Journal Watch

A scan of recent medical literature identified these articles of special importance in the science and clinical application of blood and marrow transplantation.

Weisdorf D, Bishop M, Dharan B, et al: Autologous versus allogeneic unrelated donor transplantation for acute lymphoblastic leukemia: comparative toxicity and outcomes. Biol Blood Marrow Transplant 8:213-220, 2002.

Bone marrow transplantation can yield extended disease-free survival for patients with high-risk or relapsed acute lymphoblastic leukemia (ALL). In the absence of a related, histocompatible donor, the alternatives for such patients are the use of autologous or unrelated donor marrow. The outcomes of these two treatment options were compared in a 10-year retrospective study.

The analysis included 712 patients undergoing bone marrow transplantation for ALL in first or second complete remission. Five hundred seventeen received unrelated donor marrow and 195 received autologous marrow. The outcomes of the two groups were compared in terms of engraftment, transplant-related mortality, relapse, and survival.

Median age was 14 years in the unrelated donor group vs 18 years in the autologous group; all patients were less than 50 years old. About 40% of both groups were transplanted in first complete remission. However, patients in the unrelated donor group were more likely to have high-risk characteristics, including karyotype (25% vs 13%) and white blood cell count of 50 x 10^9/L or greater (33% vs 14%).

Engraftment was similar between the two groups, although the use of ex vivo purged autologous marrow was associated with delayed engraftment. Transplant-related mortality was 42% in the unrelated donor group vs 20% in the autologous group. For patients treated in first complete remission, the relapse rate was 14% in the unrelated donor group vs 49% in the autologous group. For those in second complete remission, relapse rates were 25% vs 64%, respectively. For patients treated in first complete remission, 3-year survival was 51% in the unrelated donor group vs 44% in the autologous group; in second complete remission, the figures were 40% vs 32%, respectively. On multivariate analysis, predictors of disease-free survival beyond 6 months were unrelated donor vs autologous marrow in younger patients, transplantation in second complete remission vs first complete remission longer than 1 year, white blood cell count less than 50 x 10^9/L, performance status of 90% or greater, and transplant performed after 1995.

For patients with high-risk or relapsed ALL, transplantation with either unrelated donor or autologous bone marrow offers a chance of extended survival. The use of unrelated donor marrow is associated with a lower risk of relapse but a higher risk of transplant-related mortality. Increasing survival in this group of patients will require improvements in the safety of allogeneic transplantation and in patient selection criteria.

Vose JM, Sharp G, Chan WC, et al: Autologous transplantation for aggressive non-Hodgkin's lymphoma: results of a randomized trial evaluating graft source and minimal residual disease. J Clin Oncol 20:2344-2352.

For patients with relapsed, chemotherapy-sensitive, aggressive non-Hodgkin's lymphoma (NHL), treatment consists of high-dose chemotherapy plus hematopoietic stem cell transplantation (HSCT). There is some evidence that outcomes are better when the source of the cells is peripheral blood, rather than autologous bone marrow. The effects of HSCT source on the outcomes of high-dose chemotherapy for aggressive NHL were evaluated in a randomized, controlled trial.

The study included 105 patients scheduled to undergo autologous HSCT for high-risk, persistent, or relapsed NHL. Of these, 93 were randomized to undergo mobilized peripheral blood stem cell transplantation (PBSCT) or cytokine-naïve autologous bone marrow transplantation (ABMT). The median patient age was 47 years, with most patients considered to have a good prognosis. The same conditioning regimen—consisting of carmustine, etoposide, cytarabine, and cyclophosphamide—was used in both groups. In the PBSCT group, mobilization included granulocyte colony-stimulating factor (G-CSF), 10 µg/kg/d. In addition, both groups received G-CSF 5 µg/kg/d until neutrophil engrafment occurred. Engraftment, response rates, and other outcomes were compared between the PBSCT and ABMT groups.

For all cell lineages studied, time to engraftment was significantly shorter in the PBSCT group. Median time to an absolute neutrophil count of 500/µL or greater was 10 days with PBSCT vs 13 days with ABMT. The use of PBSCT was also associated with a shorter time to a platelet count of greater than 20,000/µL untransfused, median 11 vs 15 days; and a shorter time to independence from red blood cell transfusion, 8 vs 16 days.

Seventy-two percent of patients undergoing PBSCT achieved a complete response, compared with 54% of those undergoing ABMT. Mortality within 100 days after transplantation was 6% in the PBSCT group vs 2% in the ABMT group. Event-free survival was 37% in both groups, but overall survival was better with PBSCT: 61% vs 43%. Event-free survival was lower for patients with molecular evidence of minimal residual disease in their HSCT harvest.

The source of HSCT appears to have a significant impact on outcome for patients with aggressive NHL. Compared with ABMT, PBSCT is associated with faster neutrophil engraftment and shorter durations of transfusion dependence. Rates of complete response and event-free survival are similar between the two approaches.

Rossi S, Blazar BR, Farrell CL, et al: Keratinocyte growth factor preserves normal thymopoiesis and thymic microenvironment during experimental graft-versus-host disease. Blood 100:682-691, 2002.

Successful bone marrow transplantation relies on peripheral T-cell reconstitution, which in turn requires generation of new T cells in the thymus. The normal processes of T-cell maturation and selection may be adversely affected by graft vs host disease (GVHD), which adversely affects the function of the thymic stroma. A mouse model of GVHD was used to assess the use of keratinocyte growth factor (KGF) to prevent damage to thymic epithelial cells (TECs).

The study used a nonconditioned murine parent → F1 haploidentical transplant model, in which induction of GVHD occurs in the absence of radiation damage to TECs. Recipients were treated with KGF before or after the development of GVHD, and the effects on thymic microenvironment were assessed.

Two weeks after transplantation, the thymuses of animals receiving KGF were of normal size, cellularity, and thymocyte phenotypes, compared with saline-treated mice. This was so whether KGF was administered 3 days before or 3 days after GVHD induction. Treatment with KGF also avoided the typical GVHD-related disruption in the normal cell cycle progression of pro- and pre-T cells. Although treated animals showed a higher number of mature donor T cells, this was not significantly related to the presence of normal thymic phenotype and function. On detailed analysis TEC populations in the thymic cortex and medulla, the stromal architecture of KGF-treated mice was near normal. Expression of the KGF receptor was found exclusively on TECs, suggesting an indirect effect on thymopoiesis.

In this murine transplant model, treatment with KGF appears to have a significant cytoprotective effect on TECs, permitting continued normal T cell lymphopoiesis even in the presence of acute GVHD. Measures to increase endogenous KGF production could be a useful adjunct for the treatment of GVHD after allogeneic bone marrow transplantation.

2004 Tandem BMT Meetings Will Be Feb. 13-17 in Orlando

The combined annual meetings of ASBMT and the International Bone Marrow Transplant Registry/Autologous Blood and Marrow Transplant Registry (IBMTR/ABMTR) will be Feb. 13-17 at the Coronado Springs Resort in Orlando.

The abstract deadline is Oct. 20.

Scientific Program

Recent advances in the broad field of cellular therapy and blood and marrow transplantation will be addressed in plenary sessions, concurrent sessions, workshops, poster sessions and symposia. Topics include:

Treatment of Hematologic Malignancies
- Acute lymphoblastic leukemia
- Acute myelogenous leukemia
- Chronic myelogenous leukemia
- Multiple myeloma

Graft-vs-Host Disease
- Animal models
- New therapies

Issues in Analyzing Transplantation Data
- Medical decision making
- Biostatistical techniques

Management of Transplant-Related Complications
- Cellular therapy for infectious disease
- Monitoring and treating late effects

Transplantation Biology
- Tolerance induction/regulatory T-cells
- Posttransplantation immune reconstitution
- Minor antigens
- Chemokines and mechanisms of mobilization
- HLA and alternative donor transplantation
- Natural killer cells

Hematopoiesis
- Multipotent stem cell biology
- Mesenchymal stem cells

Gene Therapy

Tumor Vaccines

Health Care Resources

Related Conferences

In addition to the five days of scientific sessions for BMT clinicians and investigators, there will be five related conferences and courses:

- BMT Pharmacists (Feb. 12-13)
- Clinical Research Associates Data Management (Feb. 12-14)
- BMT Center Medical Directors (Feb. 13)
- BMT Center Administrators (Feb. 14-15)
- Oncology Nursing (Feb. 15-17)

Conference Chairs

The scientific program chair for ASBMT is Robert Negrin, MD, Stanford University, and the co-chairs for the IBMTR/ABMTR are Richard Champlin, MD, M.D. Anderson Cancer Center, and Olle Ringden, MD, PhD, Huddinge University Hospital in Stockholm, Sweden.

Online Registration

Online meeting registration and abstract submission will open on Aug. 1 at the ASBMT Web site at *www.asbmt.org*.

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