Prevention of Cytomegalovirus Disease in Hematopoietic Stem Cell Transplantation

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Prevention of cytomegalovirus (CMV) infection after hematopoietic stem cell transplantation (HSCT) generally involves preemptive treatment of recognized infection before the onset of overt CMV-associated disease. The success of this method depends on efficient recognition of infection and intervention before the disease progresses. Reliable tests for such diagnosis include blood culture, antigenemia assays, polymerase chain reaction assays, and other DNA sequence– or RNA sequence–based assays. For selected high-risk patients, such as patients receiving T cell–depleted hematopoietic stem cell transplants, prophylactic use of antiviral agents before the onset of CMV infection is recommended. The ability to monitor the immunological status of the patient relative to CMV-specific immunity is increasing in importance. Ultimately, the solution to the problem of efficient prevention of CMV infection in this population will require combined antiviral chemotherapy and improved reconstitution of CMV immunity.

RISK-ADAPTED ANTIVIRAL STRATEGIES FOR CYTOMEGALOVIRUS (CMV) INFECTION

Prevention strategies. Antiviral approaches to the treatment of cytomegalovirus (CMV) infection involve 2 strategies: (1) patients with documented CMV infection are treated preemptively with either ganciclovir or foscarnet, and (2) all at-risk patients are treated for a defined period with these same agents. In the first approach (the use of preemptive therapy), the antiviral agents are used only in those patients who have had CMV infection diagnosed as part of virus surveillance monitoring, in an attempt to spare a significant proportion of the population that undergoes hematopoietic stem cell transplantation (HSCT) from exposure to potentially toxic antiviral chemotherapy [1–3]. The second approach is termed “general prophylaxis”; this is used when select patients can be defined as at particularly high risk for CMV reactivation and disease, usually because of receipt of concomitant immunosuppressive treatment without the need for virus surveillance [4].

Routine dosing schedule. The dosages for available antiviral agents are shown in table 1. In general, ganciclovir and foscarnet are administered for 1–2 weeks, or until the CMV load and/or antigenemia levels begin to decrease during the induction of antiviral effect; then, a maintenance period of therapy occurs. The maintenance period varies at different transplant centers, with some providing treatment for ≥6 weeks, until immunosuppression wanes [6], and others providing treatment only for an additional 2 weeks, or until the CMV DNA load reverts to negative [7]. The dosage of ganciclovir is 5 mg/kg iv twice per day during the induction period and 5 mg/kg per day for 5 days per week during a maintenance period. When foscarnet is used, the dosage is 60 mg/kg twice per day for induction and either 60 mg/kg twice per day or 90 mg/kg per day during the maintenance period [8, 9]. If CMV relapse occurs after maintenance therapy ends, patients are usually re-treated as before for an additional 2 weeks, and the virus is usually not resistant to additional therapy [9]. Because the virus load can increase initially [9], induction therapy should be continued until there is evidence of reduction in virus load. After maintenance therapy ends, ~30%–50% of patients can be expected to have CMV relapse, especially after receipt of short-term therapy.

In the past, foscarnet has been used primarily when there is concern that the marrow toxicity of ganciclovir will threaten the graft, but foscarnet is now considered a first-line agent in preemptive therapy for CMV infection. When first used in

Table 1. Antiviral agents used to prevent CMV infection after hematopoietic stem cell transplantation.

<table>
<thead>
<tr>
<th>Antiviral Agent</th>
<th>Induction Period</th>
<th>Maintenance Period</th>
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<tbody>
<tr>
<td>Ganciclovir</td>
<td>5 mg/kg iv twice per day</td>
<td>5 mg/kg per day for 5 days per week</td>
</tr>
<tr>
<td>Foscarnet</td>
<td>60 mg/kg twice per day</td>
<td>60 mg/kg twice per day or 90 mg/kg per day</td>
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Clinical Infectious Diseases 2002;35:999–1004
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Table 1. Cytomegalovirus (CMV) prophylaxis in hematopoietic stem-cell transplant recipients.

<table>
<thead>
<tr>
<th>Prophylaxis</th>
<th>Treatment</th>
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<tr>
<td>Preemptive</td>
<td>Treat all recipients with documented CMV infection in blood or lung; for PCR assays, require 2 positive results within a 3–7-day period, unless a highly sensitive assay is used (e.g., quantitative PCR).</td>
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| Induction therapy | Ganciclovir, 5 mg/kg iv b.i.d. for 7–14 days<sup>a</sup>  
Foscarnet, 60 mg/kg iv b.i.d. for 7–14 days<sup>b</sup> |
| Maintenance therapy | Ganciclovir, 5 mg/kg iv q.d. 5–6 days/week, or 1 g po t.i.d. (adults) or 30 mg/kg po t.i.d. (children)  
Foscarnet, 60 mg/kg b.i.d. or 90 mg/kg q.d.<sup>a</sup> |
| Duration        | Duration of 2–5 weeks, on the basis of (1) time to significant reduction in immunosuppression (long course) or (2) time to return of CMV infection indicator to negative (short course). |
| General prophylaxis | Treat all HSCT recipients at highest risk for CMV infection on the basis of immunosuppressive regimen |
| Dosage          | Ganciclovir, 5 mg/kg b.i.d. for 7 days, then q.d. 5–6 days/week<sup>c</sup>  
Foscarnet, 60 mg/kg b.i.d. for period of prophylaxis<sup>a</sup> |

**NOTE.** Modified from [5]. Doses must be adjusted for renal insufficiency. HSCT, hematopoietic stem-cell transplantation.

<sup>a</sup> If no maintenance therapy is used, the induction period continues until the CMV detection assay result becomes negative. Note that the CMV load can initially increase and treatment should continue until the CMV load is negative.

<sup>b</sup> Patients should be prehydrated with normal saline before foscarnet is provided, and the Mg<sup>2+</sup> and Ca<sup>2+</sup> levels in serum should be maintained with appropriate supplements.

<sup>c</sup> Start therapy when the absolute neutrophil count is neutrophils/L, and stop therapy when the immunosuppression is significantly reduced or the absolute neutrophil count is < 1000 x 10<sup>9</sup> neutrophils/L for 2 consecutive days.

patients who had undergone HSCT, foscarnet was associated with adverse events, such as nephrotoxicity, hypocalcemia, and hypophosphatemia [10, 11]. However, with adequate hydration and electrolyte balance, safe use of foscarnet can be anticipated [7, 8, 12]. As such, foscarnet is usually administered intravenously with 2-h infusions with concomitant administration of saline, 500 mL/m<sup>2</sup>. Mg<sup>2+</sup>, PO<sub>4</sub><sup>2-</sup>, and Ca<sup>2+</sup> levels must be carefully assessed, and it is often necessary to provide an added infusion of magnesium sulfate (2 g/L) and potassium phosphate (30 mEq/L) with oral calcium carbonate supplementation if hypocalcemia occurs.

CONSIDERATION OF OTHER ANTIVIRAL AGENTS FOR CMV PREVENTION

In addition to parenteral formulations of ganciclovir and foscarnet, the other treatment options that use anti-CMV agents include acyclovir, valacyclovir, the oral formulation of ganciclovir, valganciclovir, and cidofovir.

**Acyclovir and valacyclovir.** It is recognized that, despite its relatively low potency as an anti-CMV agent, acyclovir can alter the natural course of CMV reactivation after HSCT, and it can influence the subsequent course of disease and even mortality [13–15]. The effect of acyclovir on improved survival is not understood, but it is postulated that a panherpes viral effect could result in this improved outcome [14]. However, acyclovir fails to prevent CMV disease in patients who have undergone autologous HSCT [16], and, in a retrospective review of the use of short-term, high-dose acyclovir therapy before engraftment in patients who had undergone allogeneic HSCT, acyclovir did not influence survival rates [17]. Thus, acyclovir is currently not generally used to prevent CMV infection in patients who have undergone HSCT. Valacyclovir, the improved oral formulation of acyclovir, has recently been demonstrated to be superior to orally administered acyclovir for maintaining CMV suppression, and this drug (2 g q.i.d.) may have a role in CMV prevention [18].

**Orally administered ganciclovir and valganciclovir.** Despite its poor oral bioavailability, orally administered ganciclovir (1000 mg t.i.d.; pediatric dosage, 30 mg/kg q8h) achieves levels in blood that are sufficient to suppress CMV infection [19, 20]. Use of oral ganciclovir during maintenance-phase preemptive therapy has been successful, with no change in the marrow-suppressive toxicity of the agent (R. Spielberger, personal communication). Valganciclovir is now available with improved oral bioavailability, but it has not been approved for use in patients who have undergone HSCT. Nevertheless, on the basis of experiences involving liver transplantation, a dosage of 900 mg once per day produces blood-level drug exposure (area under the curve) similar to the standard intravenous dose of ganciclovir of 5 mg/kg [21]. Studies are actively investigating the role for this agent in the treatment of patients undergoing HSCT (M. Boeckh, personal communication).

**Cidofovir.** Cidofovir is approved for the treatment of CMV retinitis but not for the prevention of CMV infection after HSCT. However, it has been used with success for preemptive...
management of CMV in patients who have undergone HSCT [22]. The dosage for cidofovir is usually 5 mg/kg per dose in 2 doses given 1 week apart, followed by maintenance therapy provided every other week, and the drug is given with probenecid and hydration. Cidofovir is nephrotoxic, and its use in patients who have undergone HSCT remains as a second-line agent when preemptive therapy with ganciclovir or foscarnet fails.

SPECIFIC CLINICAL SITUATIONS

Matched allo genic HSCT. In general, recipients of hematopoietic stem cell transplants from matched sibling and matched unrelated donors do well with preemptive strategies of treatment. However, when the risk of severe graft-versus-host disease is unusually high, anti-CMV therapy should be used early after transplantation, and it should be prophylactic rather than preemptive [3, 4, 6, 23]. Recipients of ex vivo or in vivo T cell depletion who receive an antilymphocyte globulin are also at high risk for CMV disease [24]. Also, conditioning regimens, such as fludarabine-containing regimens, have also been associated with significant rates of CMV-associated disease and should be considered for prophylactic treatment [25]. As more-aggressive conditioning regimens are developed, it will be important to recognize patients who are at particularly high risk and to provide anti-CMV treatment.

Nonmyeloablative HSCT. The risk for CMV-associated disease does not appear to be lower with nonmyeloablative HSCT or reduced-toxicity regimens [26]. The incidence of CMV reactivation is the same when nonmyeloablative conditioning regimens are used, and the occurrence of CMV reactivation can actually place these patients at significant risk for CMV disease [27]. The risk of CMV infection appears to be altered with use of fractionated stem cell preparations, as indicated by the occurrence of earlier CMV reactivation after use of either CD34⁺ cell selection or ex vivo T cell depletion [28, 29].

Autologous HSCT. Because all CMV-seropositive recipients of hematopoietic stem cell transplants are at risk for CMV reactivation, disease appears to be a function of individual postimmune reconstitution after the transplantation. Thus, in patients who have received autologous transplants, there is less risk for CMV-associated disease not so much because of a lower rate of reactivated CMV infection, but presumably because of more-efficient reconstitution of immunity [30]. Nevertheless, CMV disease occurs in these patients, and, in addition to immunologic reconstitution, the risk factors in this population include receipt of heavy pre-HSCT chemotherapy, receipt of concomitant corticosteroid treatment after HSCT, and use of CD34⁺-selected peripheral blood progenitor cells [27–31]. Patients receiving autologous hematopoietic stem cell transplants who have the aforementioned risk factors should be treated in accordance with the preemptive strategy for CMV prevention.

Persons at minimal risk for CMV. There are some patients who generally do not need to be observed routinely for preemptive treatment because the risk of infection is so low relative to the toxicity of the treatment (e.g., CMV-seronegative recipients of allogeneic hematopoietic stem cell transplants from CMV-seronegative donors). In these patients, the use of leukocyte-depleted platelets and RBCs for supportive therapy is usually sufficient to prevent primary CMV infection after HSCT [32]. Nevertheless, when early CMV infections are detected in such patients, preemptive therapy is indicated.

ASSESSING IMMUNOLOGIC RISK FACTORS FOR CMV DISEASE

It has been recognized for many years that the absence of CMV-specific cytotoxic T lymphocytes is a risk factor for CMV pneumonitis after HSCT [33, 34]. On the basis of the observation of an increased incidence of disease and mortality in CMV seronegative recipients, especially when the donor is CMV seropositive, protection from severe CMV infection has been linked to donor immunity [35–37]. Recently, CMV-specific tetramer-binding assays and intracellular cytokine assays have been used to enumerate levels of cytotoxic T lymphocyte activity [38, 39]. As improved methods for quantifying CMV-specific T cell immunity become available, there will be the potential for the use of these tests in patient treatment (e.g., to determine when preemptive therapy is no longer necessary).

CHOOSING THE OPTIMUM DIAGNOSTIC CMV ASSAY

There are generally 2 types of diagnostic assays for CMV infection: virus isolation/viral antigen assays and viral DNA/RNA assays. The isolation of CMV by tissue culture generally involves use of the “shell viral culture” and has been historically the standard assay for determining the presence of CMV reactivation [40]. Although blood cultures for CMV still form the basis for patient surveillance in some health care centers, it is recognized that this is a less sensitive method for detection of CMV infection. Instead, detection of CMV antigenemia, which relies on the enumeration of peripheral blood granulocytes that contain a viral protein called CMV-pp65 in the nucleus of infected cells, has become a very effective means for detection of CMV reactivation [41, 42]. The median time to first positive assay results for both the blood culture and the antigenemia assays is ~42 days [43], and, because the median time to CMV-associated disease is ~50–60 days after HSCT, the use of these assays in the preemptive strategy presents a relatively small margin of safety. Nevertheless, the antigenemia assay has become widely used and has had considerable success [6, 41]. It is rapid, semiquantitative, relatively easy to perform, and in-
expensive. It maintains the disadvantage of the shell vial assay, however, in that it is insensitive during periods of neutropenia.

Molecular methods have been developed to monitor CMV infection in blood cells or plasma. These methods rely on quantitative or semiquantitative measurements of DNA or RNA, and they are now increasingly being used for clinical decision making. DNA is detected either by PCR assays or by gene sequence–based assays, such as hybrid capture and nucleic acid sequence–based amplification (NASBA). By use of such tests, CMV blood infection can be detected ~35 days after HSCT [44–46]. One strategy is to use 2 consecutive PCR assays within 3–7 days before the initiation of preemptive therapy [44], although, when the newer, highly sensitive quantitative PCR methods are being used, it could be acceptable to begin treatment when the first positive result is obtained showing high CMV load. It has been shown that earlier detection of CMV infection with PCR and earlier administration of treatment can both reduce the severity of CMV disease and decrease the mortality rate [3, 44]. The quantitative measurements of CMV in blood performed by real-time PCR are becoming particularly useful and can be very helpful not only for CMV diagnosis, but also for assessment of the response to treatment [47–49]. The NASBA and the hybrid capture assays [45, 46] have been shown to be useful in patients who have undergone HSCT, and they have the advantages of being commercially available and easily implemented in most clinical laboratories.

In considering which assay to use for CMV surveillance, it is likely that there is no one single test that is optimal for all medical centers. The most recent survey of tests used in US medical centers showed that 25% of the medical centers use tissue culture methods, 25% use antigenemia assays, and ~50% use PCR- or DNA/RNA-based commercial kits [50]. The choice of diagnostic tests should be based on clinical needs, as well as on the individual institutional resources and patient population. With regard to the best specimens on which these tests should be performed, however, it appears that blood is the most useful for predicting risk of disease [1, 2, 51], and plasma can be used if the PCR assay has high sensitivity [48]. Although bronchoalveolar lavage specimens were first used to demonstrate the effectiveness of preemptive management strategies involving ganciclovir, the positive predictive value of bronchoalveolar lavage specimens for CMV detection is no better than that of blood [52, 53]. The presence of CMV on throat swabs and in urine samples is less predictive of subsequent disease [2, 51].

**FUTURE APPROACHES TO CMV PREVENTION**

Although it is hoped that nontoxic and easily administered antiviral drugs will become available in the future, there are no likely candidates presently being studied in trials that might bring such improved treatments. The lesson of the AIDS experience relative to CMV is that even modest improvement in cellular immune function is sufficient to control CMV infection. In HSCT, methods for adoptive cellular immunotherapy that use ex vivo, expanded donor T cells have been described elsewhere [54]. Newer adoptive methods are being developed for expanding CMV-specific immune T cells [55, 56], and, in the future, it is possible that cellular immunotherapy will be available for recipients of hematopoietic stem cell transplants who do not reconstitute their CMV-specific immunity. Alternatively, there is an active interest in developing vaccines for CMV, and, eventually, it may be possible that active immunization methods might become available to protect the hematopoietic stem cell transplant recipient from CMV infection [57, 58].

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