

Mini-review

Prevention and management of CMV-related problems after hematopoietic stem cell transplantation

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Summary:

Prevention and management of human cytomegalovirus (CMV) infection after hematopoietic stem cell transplantation has improved substantially in the past decade. However, with this improvement, there is increased complexity in deciding which diagnostic tests, treatment strategies and immunologic assessments are optimal for different patient populations. The purpose of this review is to address certain practical problems that commonly arise and suggest a suitable approach to management that should have wide applicability.

Bone Marrow Transplantation (2002) 29, 633–638. DOI: 10.1038/sj/bmt/1703407

Keywords: cytomegalovirus; stem cell transplantation; prevention; management

The past decade has brought a significant change in the available methods for preventing and managing cytomegalovirus (CMV) infection in recipients of hematopoietic stem cell transplants (HSCT). This has resulted in a reduction in disease incidence from 20 to 30% in the early 1990s to 5–10% today.^{1,2} In the past decade, with increasing diagnostic and therapeutic tools, the number of management decisions has become more complex, and the ability to prevent early CMV disease produces new clinical problems such as virus relapse, breakthrough viremia while on antiviral therapy and occurrence of late disease. CMV antiviral therapy is used according to two strategies. Either all at-risk patients are treated for a defined period or only transplant recipients with documented CMV infection are treated. The first approach is termed 'general prophylaxis' and the second strategy is termed 'preemptive' or early antiviral therapy (see Table 1). In the 1990s the treatment complexity hinged on the decision of which of the antiviral strategies to use. Today, this choice is not as much of a problem as deciding what procedure and assays to use for detecting and managing the patient with actual CMV infection. The changes in management that have produced the most confusion have occurred in three areas: diagnostic assays for CMV, implementation of antiviral strategies for

new transplantation methods, and concomitant use of immunologic tests to better assess patient risk. It is important to look at why we do things the way that we do them, and the purpose of this review is to put the various management options into perspective so that rational choices can be made in the prevention of CMV in HSCT patients.

Who is at risk for CMV disease?

With preemptive CMV treatment strategies, patients are only treated with anti-CMV agents when they are demonstrated to be a high risk for CMV disease. This usually translates into recognizing those patients who have demonstrable CMV infection after HSCT. For prophylactic treatment, patients are given anti-CMV therapy irrespective of CMV infection and solely because of patient-specific characteristics. The most important of these is the pre-transplant CMV serologic status of recipient and donor (R/D). For this discussion, CMV seropositive R/D pairs are those in which either or both donor and recipient are CMV antibody positive (ie R⁺D⁻, R⁺D⁺, and R⁻D⁺). The least risk for CMV infection after HSCT occurs when both donor and recipient are CMV seronegative pre-transplant (ie R⁻D⁻). In addition to this, important risk factors for CMV disease include patient age >20 years, graft-versus-host disease (GVHD), and failure of lymphoid reconstitution post HSCT.^{3,4} In regard to risk assessment, the following questions can be raised:

- (1) Should all allogeneic CMV seropositive recipients be managed the same way with respect to preemptive ganciclovir? Answer: No. In general, matched-unrelated transplants do well with preemptive strategies of treatment and are managed the same as matched-sibling donor transplants. However, when the risk of severe GVHD is unusually high, eg in T cell depletion with anti-thymocyte globulin for matched-unrelated or haploidentical HSCT, anti-CMV therapy should be used early after transplant and prophylactically rather than preemptively. It has been shown that CMV seropositivity is a strong negative prognostic factor in this setting and that preemptive anti-CMV strategies do not always improve outcome.⁵ Similarly, during the treatment of GVHD with corticosteroid-containing regimens, it has been shown that prophylactic ganciclovir is useful.⁶

Table 1 Use of antiviral drugs for prevention of CMV disease after HSCT

Antiviral strategy	Definition	Ganciclovir dose ^a	Advantages	Disadvantages
Prophylactic use – general use	Use of anti-CMV drug in all allogeneic HSCT recipients at the time of engraftment	5 mg/kg bid for duration of risk period (eg. to d 100)	No required lab surveillance	Marrow toxicity Increased infections
Modified prophylaxis – risk adapted	Use of anti-CMV drug in those at highest risk (eg steroid use for GVHD, <i>in vivo</i> T cell depletion)	5 mg/kg bid for duration of risk period ^{b,c}	No required lab surveillance Targeted population	Marrow toxicity Increased infections
Preemptive use – risk based	Use of anti-CMV drug only in those with demonstrated CMV reactivation in blood	5 mg/kg bid for 1–2 weeks, then 5 mg/kg qd 5–6 days/week for 2–5 weeks	Targeted population Improved survival	Continuous surveillance required

^aAlternatively, foscarnet can be used at a dose of 60 mg/kg bid in persons who cannot take ganciclovir because of marrow dysfunction. Adjustments are required for kidney function-impaired patients.

^bCessation of treatment when CMV surveillance test becomes negative.

^cOral ganciclovir (1000 mg tid) can be substituted for i.v. drip during maintenance therapy.

- (2) Is the risk for CMV disease less with non-myeloablative HSCT? Answer: No. Despite the reduction in some aspects of HSCT procedure-related morbidity by non-myeloablative conditioning regimens, the incidence of CMV reactivation is approximately the same, and the occurrence of GVHD can actually place the patient at high risk for CMV.⁷ It is likely that the risk of CMV disease is the same as that observed in conventional matched-sibling donor transplantation, but large patient series have not been published.
- (3) Is the risk of CMV reactivation less with unmanipulated HSCT? Answer: Yes. Use of *in vivo* T cell depletion and selection of CD34⁺ stem cells increase the reactivation rate of CMV infection. It has been reported that time to CMV reactivation decreases by approximately 1 week for each of these manipulations – CD34⁺ cell selection and *in vivo* anti-T cell therapy.⁸
- (4) Should R⁻D⁻ patients ever be preemptively treated with ganciclovir? Answer: No. In the allogeneic HSCT setting, with the introduction of filtered blood products, CMV disease has been virtually eliminated from the CMV-seronegative recipient.⁹ This does not mean that CMV disease never occurs in the R⁻D⁻ patient, but it would not be worth subjecting this group to the surveillance needed in the preemptive treatment. Rather, CMV should be ruled out in appropriate clinical syndromes as they occur.
- (5) Should autologous HSCT recipients be preemptively treated with ganciclovir? Answer: Sometimes. There is definite but low risk of CMV disease after autologous HSCT, but unless there is a particular risk factor, preemptive ganciclovir is not worth the expense. Instead, interval evaluations should include a CMV diagnostic test for such events as fever and other problems for which CMV should be considered. Factors which increase the risk of CMV disease include heavy pre-HSCT chemotherapy, concomitant corticosteroid usage post HSCT, and use of CD34⁺-selected stem cells.^{10,11}

What are the best diagnostic options?

Preemptive treatment of CMV places a burden on the transplant team to detect CMV infection in blood before pro-

gression to overt disease.^{2,12,13} As described above, if the risk for CMV infection is sufficiently high, it might not be justifiable to wait for diagnostic evidence of CMV infection before initiating antiviral therapy.⁶ However, for the majority of T-replete allogeneic HSCT recipients, it remains safer to reserve anti-CMV treatment until there is evidence of infection.¹³

The tests for CMV are generally of two types: replication/antigen assays and DNA/RNA assays. The standard assay for presence of CMV replication is the shell vial culture.¹⁴ Detection of CMV antigenemia relies on the enumeration of peripheral blood granulocytes containing a viral protein called CMV_{pp65} in the nucleus of infected cells.¹⁵ In HSCT recipients, the antigenemia assay and the shell vial culture become positive at a median time after transplant of approximately 42 days.¹⁶ Since CMV-IP occurred at a median time of 50–60 days post HSCT in the pre-antiviral era, the use of the culture or the antigenemia assays present a relatively small margin of safety for negative assays. Nevertheless, the antigenemia assay is a standardized one which has become widely used and with success.¹⁵ Its advantages are that it is rapid, fits well into laboratory systems, is semi-quantitative and inexpensive. Historically, this assay was an improvement on blood cultures (shell vial assay¹⁴) but, like the culture assay, it has the disadvantage that it requires that there be no neutropenia and that infection has progressed sufficiently to be detectable in granulocytes.

The direct detection of CMV DNA in blood cells or plasma has improved with the ability to monitor CMV infection. With these molecular methods of CMV detection, infection will generally be detected earlier than with replication/antigen assays. DNA is detected either by PCR assays or by hybrid-capture assays and can detect CMV blood infection at approximately day 35 post HSCT.^{12,17} Thus, the DNA-based assays widen the opportunity for early treatment by approximately 1 week. In a study in which patients were randomly managed with PCR vs standard blood culture, there was not only earlier implementation of preemptive ganciclovir but also significantly less disease and less overall mortality.¹² In addition, PCR can be used for following the response to treatment.¹⁸ The hybrid capture assay is a non-amplification-based DNA assay for detection of CMV and is effective clinically for detection of CMV after HSCT.¹⁷

Nucleic acid sequence-based amplification (NASBA) is a molecular assay that detects CMV replication using an RNA amplification technique.¹⁹ The principle of the NASBA assay is that a specific RNA molecule can be made into DNA, and this can serve as a template for amplification of signal, resulting in improved detection. A potential advantage of both the hybrid capture assay and of NASBA is that they are commercially available and readily implemented into most clinical laboratories. The availability of these new diagnostics raises some questions:

- (1) CMV detection in blood, bronchoalveolar lavage (BAL), throat, or urine – which patient specimen is most useful for predicting risk of disease? Answer: CMV detection in blood. Although BAL is an early site of CMV reactivation after HSCT, the positive predictive value of BAL is no better than CMV detection in blood.^{20,21} Because approximately 40% of allogeneic HSCT recipients will have asymptomatic CMV infection in BAL at 1–2 months after transplant,²² quantitative measurement of CMV DNA/RNA in blood should become the most predictive assay for subsequent disease.^{23,24} Throat infection is less reliable than blood and BAL and urine is non-predictive of subsequent disease.^{3,25}
- (2) Considering CMV culture, antigenemia assay, PCR, hybrid capture assay, and NASBA – which is best for HSCT patient management? Answer: There is no one best test. The choice of diagnostic test must be made based on clinical needs, individual institutional resources and the patient population. All of the CMV tests mentioned can be used alone for adequate management of selected populations.^{12,15,17} Even the shell vial culture can be sufficient when the population has a high rate of anticipated pulmonary infection.²⁶
- (3) What is the best way to follow a patient while on treatment? Answer: DNA-based test. It is recognized that CMV breakthrough can occur in up to 50% of post-HSCT patients while on ganciclovir.¹⁶ Because such virus breakthrough could be a sign of drug-resistant virus, it is important to assess the infection quantitatively, and a DNA-based assay is best for this. The disadvantage of the quantitative PCR assay is that there is no standardized assay that is readily available, the assay performed by commercial diagnostic services is expensive, and PCR remains positive for a longer time period. Compared to the antigenemia assay, which returns to negative values at a mean of 2.5 weeks post treatment, the PCR assay remains positive for a mean of 4.4 weeks of treatment.¹⁶ Thus, the antigenemia assay has great versatility in both predicting risk for disease and in monitoring infection. However, the quantitative measurement of CMV DNA in blood is useful for assisting in detecting progressive infection while on treatment. Eventually, when generally available, it is likely that CMV DNA detection with ‘real time’ PCR will permit efficient assessment of infection and could eventually become the preferred assay for management of CMV after HSCT.^{23,24}
- (4) Is there any use for a CMV IgG or IgM antibody assay in post-HSCT management? Answer: No.

Which anti-CMV drug is optimal after HSCT?

There are four antiviral agents approved for use in CMV infection — ganciclovir (valganciclovir), acyclovir (valacyclovir), foscarnet and cidofovir, and each has a role in the management of HSCT patients. Most CMV antiviral drug usage is directed at prevention of disease but treatment of disease remains only variably successful in the HSCT recipient.

Ganciclovir

The prevention of CMV disease with intravenous ganciclovir was first shown in studies after HSCT.^{22,25,27} As noted above, patients at highest risk for CMV disease are treated with prophylactic ganciclovir which is normally started at the time of engraftment (absolute neutrophil count = $750 \times 10^{-9}/l$) and continued to the point of reduced immunosuppression, approximately 100–120 days post transplant. A randomized controlled trial of prophylaxis²⁷ demonstrated a significant reduction in CMV excretion (3% vs 45%) and CMV-associated disease (10% vs 29%), but no reduction in mortality. This lack of effect of ganciclovir on mortality was different from the effect observed with preemptive use of ganciclovir in the same population.²⁵ With preemptive therapy, ganciclovir is started at the time of CMV infection at a dose of 5 mg/kg i.v. given twice daily for 7 days and then 5 mg/kg once daily for 5–6 days per week for 2–5 additional weeks (see Table 1). Oral ganciclovir can be substituted for i.v. drug during maintenance therapy and is used at a dose of 1 g three times daily, although it is not approved for this use in transplant patients. Valganciclovir is also commercially available and, because improved absorption of this drug can produce blood levels similar to i.v. ganciclovir, this formulation is being evaluated for use in HSCT.

Whether used before or after transplant, prophylactic ganciclovir is associated with marrow toxicity, and drug-related neutropenia occurs in approximately 30% of transplant recipients receiving ganciclovir.^{25,27,28} The median time of onset of neutropenia is 36 days (range 6–74 days) after starting treatment, and the neutropenia persists for a median of 12 days (range 4–20 days).^{25,28} As noted, in addition, the use of prophylactic ganciclovir has been associated with the failure of development of CMV-specific cellular mediated immunity and an increase in late CMV-associated disease.²⁹

Acyclovir

Despite a relatively low potency against CMV *in vitro*, acyclovir can perturb the natural course of CMV reactivation after HSCT and can have pronounced effects on clinical disease and mortality.³⁰ In groups treated either with high-dose acyclovir (800 mg four times daily) for 1 month or 6 months after marrow transplant or with a low dose acyclovir regimen (200 mg four times daily) for 1 month, significant reductions in CMV infection occurred in those receiving high-dose acyclovir either for 1 month or for 6 months.³¹ However, there was no difference in the incidence of CMV-disease among the three groups (8% vs 14%

vs 11%, respectively).³¹ However, the group that received a 6-month course of high-dose acyclovir had a significant improvement in survival. Currently, valacyclovir, an improved oral formulation of acyclovir, is being evaluated for efficacy in HSCT recipients.

Foscarnet

Foscarnet has become an important agent in the prevention and treatment of CMV infection after HSCT. Initial experiences with foscarnet indicated that nephrotoxicity, hypocalcemia and hypophosphatemia were significant problems,^{32,33} but with adequate attention to hydration and electrolyte balance safe use is observed.³⁴ For preemptive treatment, foscarnet can be substituted for ganciclovir and is used at a dose of 60 mg/kg twice daily.³⁵

Cidofovir

Cidofovir is approved for treatment of AIDS retinitis and, although it has been used in HSCT recipients, it remains a second line agent for CMV because of the associated renal toxicity.³⁶

The use of these antivirals raises several questions:

- (1) How long should preemptive ganciclovir be given? Answer: There is currently a spectrum of practice that includes the following methods of preemptive therapy with ganciclovir: (1) treat for 2–3 weeks or until a sensitive DNA test is negative and repeat if CMV relapse occurs, (2) treat for 6 weeks and monitor for relapse, (3) treat for 10 weeks. CMV relapse is seen more frequently with short courses of therapy, but toxicity and drug resistances are more frequent with longer treatment.
- (2) When should foscarnet be substituted for ganciclovir? Answer: Foscarnet should be used when marrow dysfunction prevents the continued use of ganciclovir or when progressive infection occurs while on ganciclovir with or without evidence of ganciclovir resistance. In the clinical situation, CMV resistance testing is not readily available, and clinical assessment on other grounds is usually necessary. Foscarnet should be used in patients for whom the quantitative DNA assays show rising blood levels of CMV DNA. The ability of ganciclovir to completely suppress CMV in the absence of CMV-specific immunity is limited. Thus, for example, it has been observed that antigenemia can increase in a third of patients after starting ganciclovir³⁷ and, in those on prophylactic ganciclovir, breakthrough PCR positivity occurs in >60% of patients.¹⁶ There is little evidence to suggest that this represents ganciclovir-resistant virus, but experience with solid organ recipients indicates that this could become a greater problem after HSCT.³⁸ Patients with persistence of CMV infection and unexplained clinical deterioration should be switched to foscarnet treatment. Alternatively, it has been shown that ganciclovir and foscarnet can be used on alternate days to suppress virus while lessening marrow toxicity.³⁹
- (3) How should the patient be treated when CMV infection occurs just prior to HSCT? Answer: The occurrence of CMV infection in the days just prior to HSCT need not change the post-HSCT CMV management plans. If preemptive treatment was intended, then this can proceed. However, a method of CMV detection that does not require neutrophils should be used during the pre-engraftment period. An alternative approach is to use ganciclovir at 2.5 mg/kg i.v. three times daily on days –8 to –1 pre-transplant and then resume prophylactic ganciclovir at 5 mg/kg daily for 5 days per week at the time of engraftment.⁴⁰
- (4) When should oral ganciclovir or valganciclovir be used? Answer: Oral ganciclovir is poorly absorbed but using a dose of 1 g three times daily, adequate blood levels can be achieved.⁴¹ Marrow suppression occurs as frequently with oral drug as with i.v. administration. Recently, a pilot study has suggested that oral ganciclovir can be used during the maintenance phase of preemptive ganciclovir, ie weeks 2–6 of treatment after CMV blood infection.⁴² With the availability of valganciclovir, it is possible to improve oral bioavailability but the antiviral effects and the toxicity are unlikely to be different from i.v. ganciclovir. Neither oral ganciclovir nor valganciclovir have approved indications for use in HSCT.
- (5) How should the recipient with CMV be managed late after HSCT? Answer: The patient with CMV infection >100 days after HSCT has usually had a prior infection that was managed with ganciclovir and often has either GVHD or poor marrow function or both. With relapsed CMV infection, ganciclovir treatment could worsen marrow function or even prolong a virus-specific immunodeficient state.²⁹ It is important here to modulate the immunosuppressive regimen and the antiviral drugs. Quantitative PCR can be useful to monitor CMV infection during this time. If the CMV DNA level in blood is rising foscarnet must be used.
- (6) What is the role of intravenous immune globulin in prevention of CMV disease? Answer: Not adequately known. Many clinical evaluations of IVIG for prevention of CMV disease antedate the current preemptive and risk-adjusted prophylactic antiviral chemotherapy strategies. Studies that use IVIG with preemptive ganciclovir have not clearly demonstrated a role for IVIG in prevention of CMV.⁴³
- (7) What is the best way to treat overt CMV disease? Answer: Treatment of CMV-associated pneumonitis is often unsuccessful, especially since the onset of disease is at 173 days post HSCT, at a time when months of treatment of GVHD, fungus infection, and CMV could have produced multiple organ toxicities. Ganciclovir is the recommended agent for treatment of CMV-associated disease and is used at 5 mg/kg twice daily for 21 days. For pneumonitis, it is customary to give concomitant intravenous immune globulin (IVIG) at a dose of 500 mg/kg every other day for 3 weeks. Other CMV-associated organ-specific syndromes respond better to ganciclovir treatment and do not require IVIG.

What is the future of CMV preventive therapy?

The key to better patient management is more efficient control of CMV infection during immune reconstitution after HSCT. To accomplish this, it is important to develop newer methods of immunotherapy. It has been known for years that HSCT recipients with cytotoxic T lymphocyte (CTL) activity targeted to CMV have significantly less disease than those without CTLs.^{44,45} Also, the absence of CMV-specific CD4⁺ T cell function is a marker for risk of CMV disease.^{46,47} More recently, CMV-specific tetramer-binding assays and intracellular cytokine assays have been studied in the setting of HSCT to enumerate CD8 function.^{48,49} With the development of rapid methods to quantitatively measure CMV-specific T cell immunity, there is the potential for use of these tests in patient management. It is possible that these newer assays will be able to determine the point at which monitoring for CMV is unnecessary. It appears that donor immunity to CMV is protective,⁵⁰ and methods of adoptive cellular immunotherapy using *ex vivo* expanded donor T cells have been described.⁵¹ Newer adoptive methods are being developed for expanding both CMV and Epstein-Barr virus-specific immune T cells.^{52,53} Alternatively, active immunization of donor and recipient could be possible for more efficient expansion of donor memory function in the recipient.⁵⁴ Eventually, using either passive transfer of CMV specific T cells or active immunization of donor and recipient, it will hopefully become possible to control CMV using immunotherapy.

Acknowledgements

The author expresses gratitude to Ms Suzanne Kelly for the preparation of this manuscript. The author is supported in this work by grants CA30206 and M01 RR-43 from the National Institutes of Health.

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