CMV-specific immune reconstitution following allogeneic stem cell transplantation

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ABSTRACT
Cytomegalovirus (CMV) remains a major contributor to morbidity and mortality following allogeneic haemopoietic stem cell transplant (HSCT) despite widespread use of viraemia monitoring and pre-emptive antiviral therapy. Uncontrolled viral replication occurs primarily in the first 100 d post transplant but this high risk period can extend to many months if immune recovery is delayed. The re-establishment of a functional population of cellular effectors is essential for control of virus replication and depends on recipient and donor serostatus, the stem cell source, degree of HLA matching and post-transplant factors such as CMV antigen exposure, presence of GVHD and ongoing use of immune suppression. A number of immune monitoring assays exist but have not yet become widely accessible for routine clinical use. Vaccination, adoptive transfer of CMV specific T cells and a number of graft engineering processes are being evaluated to enhance of CMV specific immune recovery post HSCT.

KEYWORDS
adoptive T cell transfer; CMV immunity; Cytomegalovirus; haemopoietic stem cell transplantation; immunotherapy

Introduction
Cytomegalovirus (CMV) is a betaherpesvirus that maintains lifelong latency after primary infection. 50 to 90% of the population are CMV seropositive, although this figure varies with age and geographic location. CMV reactivates when host immunity wanes in the context of haemopoietic stem cell transplantation (HSCT) and uncontrolled viral replication can lead to disease in a number of tissues. Despite improvements in monitoring and pre-emptive therapy with antiviral agents, CMV remains a major contributor to post-transplant infectious and overall morbidity and mortality.\textsuperscript{1} Recovery of immune function is essential to control of reactivation. Here we review what is known about CMV immunity post HSCT, how it can be measured and interventions that may facilitate more rapid immune recovery.

The clinical impact of CMV post-allogeneic HSCT
In recipients of HSCT, CMV is one of the major pathogens responsible for infectious morbidity and mortality. Viraemia usually precedes clinically significant tissue injury. Uncontrolled viral proliferation can cause pneumonitis, gastritis, enteritis, colitis, hepatitis, bone marrow suppression, retinitis and encephalitis.\textsuperscript{1} CMV disease is defined as the presence of symptoms and signs consistent with CMV end organ infection together with detection of the virus by a validated method including immunohistochemistry or viral culture. PCR positivity in tissue without other evidence of disease is not considered diagnostic of CMV disease.\textsuperscript{1} CMV infection in HSCT occurs primarily due to reactivation of latent infection in recipients who are seropositive pre transplant. Primary infection of seronegative HSCT recipients occurs largely due to infusion of a stem cell product from a seropositive donor. Transfusion related primary infection is now rare due to the introduction of screening for CMV serostatus of blood donors and the use of leucodepleted blood products.\textsuperscript{2} The majority of CMV reactivation is observed in the first 100 d post transplant, but late CMV has also become more frequent as a result of conditioning regimens or graft vs. host disease that produce prolonged immune suppression, and prophylactic or pre-emptive therapy that delay immune reconstitution.\textsuperscript{3}–\textsuperscript{6}

Surveillance for viral reactivation and pre-emptive therapy with ganciclovir or foscarnet for asymptomatic viraemia is the most widely accepted approach to management of CMV post HSCT. Therapy is instituted with the aim of limiting viral replication prior to the development of organ damage. Routine surveillance for the presence of CMV viraemia is performed regularly during the
period of high risk up to 100 days, and extended in cases of GVHD and ongoing immunosuppression. Ganciclovir used at the onset of detectable viraemia has been shown to significantly reduce the incidence of CMV disease at 100 d when compared with placebo.\textsuperscript{7} In clinical practice the threshold viral load to trigger antiviral therapy may vary depending on the presence of risk factors such as the type of transplant, the use of corticosteroids or presence of GVHD and physician preference. Threshold triggers are not currently standardised and it is not known whether withholding antiviral therapy may be safe in some subgroups of patients with CMV reactivation such as those with measurable CMV specific cellular immunity. A recent single center retrospective analysis of a CMV monitoring and pre-emptive therapy regimen in 926 transplants with seropositive donors or recipients demonstrates the ongoing burden of CMV. Post-transplant CMV was monitored using quantitative PCR and a treatment threshold viral load of 150IU/ml (using the WHO standard). The CMV reactivation rate was 69\% in the first 100 d. Pre-emptive antiviral pharmacotherapy was initiated in almost all these patients but progression to high viral titer (>500 IU/ml) and CMV disease was not prevented in all patients. The cumulative incidence of CMV disease was 11\% at one year. The direct CMV disease mortality was low (1\%) but overall mortality in patients who reactivated was higher than in those that did not.\textsuperscript{8} The use of antiviral agents as a pre-emptive strategy or as treatment in such a large proportion of patients comes with significant draw-backs. It is ineffective in some cases due to viral resistance.\textsuperscript{9,10} Ganciclovir is myelosuppressive while foscarnet is nephrotoxic and causes renal tubular acidosis and electrolyte disturbance.\textsuperscript{11} The burden of cost of monitoring and treatment with currently available antivirals is high. The overall increment of cost of CMV reactivation compared to no reactivation is estimated in the tens of thousands of dollars.\textsuperscript{12}

Widespread adoption of surveillance and pre-emptive treatment has led to a fall in the number of patients progressing to CMV disease, most commonly pneumonitis which still has a fatality rate of 50\%, from 70--90\% prior to the availability of antiviral pharmacotherapy.\textsuperscript{4,13-15} Nonetheless, patients who are seropositive for CMV or who receive transplants from CMV seropositive donors continue to suffer an excess of non-relapse mortality after transplant.\textsuperscript{16-21}

Given the impact of CMV post transplant, measures aimed at prevention and early detection of CMV in HSCT are warranted. Primary prevention includes the early assessment of recipient CMV status, with the use of leukodepleted blood products for all seronegative recipients. Due to the impact on transmission and risk of CMV infection, as well as CMV-specific immune reconstitution (as discussed later), CMV status is an important consideration in HSCT donor selection, particularly in unrelated donor transplantation. Guidelines recommend prioritising CMV seronegative donors for CMV seronegative recipients, and CMV seropositive donors for CMV seropositive recipients.\textsuperscript{19,21,22}

There is a clear need for alternative therapies for CMV. A number of pharmaceutical alternatives are under investigation. Brincidofovir, a lipid conjugated produrg of cidofovir, showed strong antiviral activity in preclinical and early phase clinical trials. While a significant reduction in the incidence of CMV was shown in a phase III trial, the therapeutic window was narrow with unacceptable gastrointestinal side effects seen at higher dosages.\textsuperscript{23} Maribavir, a benzimidazole antiviral agent that inhibits viral replication, did not meet the endpoint of prevention of CMV disease.\textsuperscript{24} Letemadlovir demonstrates dose dependent antiviral activity and a favorable safety profile and a phase III study is underway.\textsuperscript{25}

**CMV immunity in the normal individual**

CMV infection results in the development of an adaptive immune response involving humoral and cellular factors. The relative importance of humoral immunity in humans is not clear, with conflicting indirect evidence. Clinical observations imply that humoral immunity at best plays only a part in the control of CMV. The presence of high titer antibodies has been associated with protection from neonatal transmission of the virus\textsuperscript{26,27} and with improved outcomes in HSCT recipients with CMV reactivation in one study,\textsuperscript{28} but not in another.\textsuperscript{29} Hyperimmune globulin used to treat pregnant women with primary CMV infection improves foetal outcomes and abrogates placental pathology,\textsuperscript{30,31} and is used in solid organ transplant to reduce primary infection. Use of hyperimmune CMV globulin in HSCT recipients is not supported by strong evidence. Nonetheless, it is commonly administered as an adjunct to ganciclovir in pneumonitis where mortality remains high.\textsuperscript{32}

There is overwhelming clinical evidence of the central role of the cellular immune system in defense against CMV. Cellular immune deficiency and predisposition to CMV infection is seen in HSCT recipients,\textsuperscript{1} advanced HIV infection\textsuperscript{33} and in patients treated with lympholytic chemotherapy such as the purine analogs (fludarabine and cladribine) or the anti-CD52 antibody alemtuzumab.\textsuperscript{34-36} Reconstitution of CMV cell mediated immunity via adoptive transfer of CMV specific immune effectors has been shown to control CMV infection in both animal models\textsuperscript{37,38} and human trials.\textsuperscript{39-47}
In otherwise healthy individuals, both CD8\(^+\) and CD4\(^+\) cells targeting multiple CMV peptides are important in the control of infection. The proportion of the immune response devoted to CMV increases with age in seropositive individuals. CMV specific cells comprise up to 10% of circulating CD8\(^+\) cells in seropositive individuals,\(^{58}\) a disproportionate allocation given the large number of pathogens to which the immune system must respond. The immunodominant proteins pp65, pp50, glycoproteins IE-1 and IE-2 account for the majority of the T cell repertoire and subdominant responses are present to other CMV proteins including glycoprotein-H and pp28.\(^{49-51}\) CD8\(^+\) cells recognize epitopes of CMV proteins in a predictable manner that is HLA determined. The major tegument protein pp65 and immediate early protein-1 (IE-1) are the most extensively studied immune targets in HSCT recipients.

CD4\(^+\) cells also play a major role in the control of CMV. Target proteins include pp65, glycoproteins B and H, IE72, IE86 and UL69.\(^{52,53}\) Evidence of the importance of CD4\(^+\) cells can be derived from clinical situations in which CD4\(^+\) lymphopenia is present, such as HIV infection\(^33\) and in renal transplant recipients where CD4\(^+\) lymphopenia is particularly associated with CMV reactivation.\(^{54}\) There is evidence that CD8\(^+\) CMV specific cells are insufficient to control CMV in the absence of CD4\(^+\) cells.\(^{55,56}\)

**CMV immunity in HSCT recipients**

In the HSCT population, measurable T cell recovery occurs within the first few months of transplant. Risk from CMV is highest in the first 100 d when immunity is in the process of recovery after exposure to lympholytic agents in pre-transplant chemotherapy and transplant conditioning regimens, and in some cases T cell depletion, either \emph{ex vivo} or \emph{in vivo}. The number and quality of T cells transferred in the stem cell graft varies with the source (peripheral blood stem cells > bone marrow > cord blood). In patients undergoing spontaneous immune recovery post HSCT both CD8\(^+\) and CD4\(^+\) T cell subsets recover together, and are quantitatively correlated with control of CMV reactivation.\(^{57}\) Adoptive immunotherapy trials support the assertion that both CD4\(^+\) and CD8\(^+\) cells are important. Infusion of CD8\(^+\) clones was effective in clearing CMV, but cells did not persist in patients who did not develop a CMV specific CD4\(^+\) response.\(^{58}\) Einsele demonstrated that by infusing CD4\(^+\) cells alone, CD8\(^+\) cells were generated \emph{in vivo}.\(^{59}\)

Lack of recovery of immune function in the context of reactivation is associated with prolonged CMV viraemia and adverse outcomes.\(^{57,60-62}\) This is impacted by donor serostatus (faster with D+), donor source (faster with matched related bone marrow, slowest with cord blood transplant), degree of match (slower with mismatch) and conditioning regimen (slower with T cell depletion). Numeric recovery of CMV specific immunity is not sufficient to control viral replication. The capacity to produce multiple cytokines and establish longevity in the recipient (compartmentalised according to functional memory subsets) is also required.\(^{63,64}\)

The reactivating CMV virus strains are generally of recipient origin, and control is mediated by donor derived immune effectors.\(^{65-67}\) This explains the differential risk according to donor and recipient serotype. The highest risk group is seropositive recipients (R+) with seronegative donors (D−) in which reactivation occurs in up to 80% of cases. R+/D+ are at moderate risk, R−/D+ at lower risk, with reactivation rate less than 10%. Primary infection in R−/D− transplants is rare.\(^{19,60,68-70}\)

In D−R+ scenarios immune recovery is slower but does occur, with evidence that immune recovery is mediated by naive donor T cells derived from progenitors in the graft.\(^{56}\) It has traditionally been held that T cells from the transplant donor are the sole source of CMV control as recipient immune effectors are ablated by the transplant process. This may be the case for myeloablative transplants but there is evidence that in stem cell transplants conditioned with reduced intensity protocols recipient CMV specific T cells contribute to CMV immunity, particular early after transplant before achievement of full lymphoid chimerism.\(^{71,72}\)

**Factors influencing CMV immunity post transplant**

The immune recovery process is dynamic and influenced by post-transplant events. There is a bidirectional relationship of viraemia to immune recovery. While immune recovery is required for control, the presence of antigen stimulation is required to stimulate clonal proliferation of antigen specific T cells. Seronegative recipients are less likely to develop detectable immunity presumably because the lack of viral reactivation in the recipient does not provide a source of antigen stimulation. In adoptive cell therapy studies, \emph{in vivo} T cell expansion is associated with episodes of detectable antigenaemia.\(^{46}\) In the absence of GVHD, CMV specific immune recovery is stable and long-lasting. GVHD and associated treatment adversely affect immune recovery and can lead to prolonged problems with CMV\(^74,57\) (see Fig. 1).

During viral reactivation, massive \emph{in vivo} expansion of CMV specific clones occurs to the detriment of other immune subsets, such as naïve T cell subsets including recent thymic emigrants.\(^{75}\) These expanded clones are largely CMV specific terminally differentiated effector
cells as measured by gene expression analysis and TCR sequencing of single cells sorted by flow cytometry.\textsuperscript{74} Early clonal expansion produces a period of oligoclonality with a small number of CMV specific clones comprising a large proportion of all CD8\textsuperscript{+} cells early post transplant. It is possible that this represents expansion of clones transferred with the graft on exposure to antigen in the host.\textsuperscript{74} Later in the recovery process the TCR diversity expands with new clones derived from stem cell graft progenitors.\textsuperscript{66} CMV serostatus has a strong impact on the pattern of global immune recovery, such as the ratio of B cells to T and NK cells, in addition to the well known effects on T lymphocytes.\textsuperscript{76} NK cell subsets recover early post transplant and are involved in the response to CMV, as are V\delta2-negative \(\gamma\delta\)T cells.\textsuperscript{77,78} It is likely that NK cells contribute to control of CMV infection with expansions of IFN-\(\gamma\) producing NK cell populations reported in response to and after resolution of CMV reactivation post HSCT.\textsuperscript{79,80}

While CMV is the most common virus to cause clinical problems post transplant, concurrent infection with more than one double stranded DNA virus is common in a number of transplant settings, particularly following cord blood and T cell depleted transplant, and is associated with adverse outcomes such as increased overall mortality.\textsuperscript{81} Reactivation of one or more viruses may

\textbf{Figure 1.} CMV immune recovery post-allogeneic HSCT. (A and B) Absence of CMV reactivation does not stimulate clonal expansion of CMV specific T cell clones and detectable CMV immunity is low or undetectable. When CMV-VSTs are administered prophylactically no expansion of the transferred clones is observed. (C and D) Low level CMV reactivation is controlled by CMV-VSTs that recover in the first few months post-HSCT. Prophylactic or pre-emptively administered CMV-VSTs are seen to expand in vivo and produce long-lasting stable immunity that is detectable up to 10 y after transplant. (E) CMV immunity recovers and controls CMV without treatment in the first few months. Subsequent development of GVHD and administration of corticosteroids and other immune suppressive medications results in loss of CMV immunity and recurrent CMV reactivation requires treatment with antiviral pharmacotherapy. (F) After failure to establish an effective cellular immune response spontaneously, either due to treatment (as in E) or donor seronegativity, donor-derived or third party banked CMV-VSTs administered therapeutically can rescue patients refractory to standard therapies.
reflect global cellular immune deficiency or may stem from the fact that CMV infection itself results in contraction of the immune repertoire post transplant thus predisposing to other viral infections. It is not possible to distinguish these possibilities using presently available information. Recovery of CMV specific immune function has been postulated as a biomarker for overall immune recovery but no causal link between this single pathogen-specific and global immune recovery has been shown.

There is a growing body of evidence that polymorphisms in the genes encoding molecules involved in the CMV-host interaction affect the incidence and natural history of CMV post transplant. These include SNP (single nucleotide polymorphisms) in the chemokine receptor 5 (CCR5), monocyte chemoattractant protein 1 (MCP-1), interleukin (IL) 10, toll-like receptors (TLR) 8 and 9, dendritic cell-specific molecule-3-grabbing non-integrin (DC-SIGN) and IL-28B genes. SNPs in these genes in donor and recipient have been associated with the occurrence of CMV reactivation, its duration, the peak levels of CMV DNAemia and the development of CMV disease. To date study findings have been inconsistent, limited by small numbers and mechanistic data is lacking. Larger confirmatory studies are required before recipient and donor SNPs can be used for risk stratification or to guide therapeutic interventions.

Detailed assessment of global immune recovery post transplant is currently underway using powerful tools such as multidimensional flow cytometry and mass cytometry. Availability of new methods for single cell analysis, gene expression and bioinformatics tools to map TCR will facilitate ever more detailed interrogation of post-transplant immune function.

**Measurement of CMV specific immune function in the clinic**

A variety of methods are available to characterize CMV specific immunity. Fluorescently-labeled MHC multimers loaded with individual immunodominant CMV epitopes which bind with high affinity to the TCR allow for specific and rapid identification of CMV specific T cells using multiparameter flow cytometry. However, this approach is limited by the HLA type, knowledge of individual epitopes, the frequency of multimer positive cells and will only give an indication of a proportion of the immune response. Multimer-based assessment provides no indication of functional responsiveness, and thus numeric thresholds may provide false reassurance of immunity in the early post-HSCT population. Functional assays include the enzyme linked immunoassay (ELISPot), cytokine production assays and cytotoxicity assays (including Chromium release, degranulation assays or direct visualization assays). ELISPot has the advantage of reproducibility and is semi-quantitative so it can show change in immune function over time. However it is limited by the lack of individual cellular phenotype information and the need to standardise assays performed at different times. Cytotoxicity assays require large cell numbers and are relevant only for research purposes. Quantitative flow cytometry assays use intracellular flow cytometry to measure production or expression of IFN-γ, TNF, CD107 and IL-2 from CMV-stimulated PBMCs; reacting cells are then quantified using absolute CD3+, 4+ and 8+ cell counts. Lilleri et al used quantitative flow cytometry to assess immune reconstitution over time in 131 patients. This study had a relatively high threshold CMV copy number for initiation of pre-emptive treatment (>30,000 copies/μl) so is helpful in understanding the natural history of CMV immune recovery in the absence of therapeutic intervention. It should be noted that the majority of patients were young recipients of bone marrow grafts and few had cord blood or T cell depleted transplants that are associated with higher risk of uncontrolled CMV infection. This study showed that patients with CMV specific immunity above a predetermined cut off (1 and 3 CMV specific CD4+ and CD8+ cells/μl, respectively) were able to control reactivation without the need for antiviral chemotherapy. The only failures were associated with treatment for GVHD. Time to development of both CD4+ and CD8+ immunity was correlated with time to control of CMV. Similar observations have been made in other studies utilizing quantitative or semi-quantitative methods for measurement of CMV immunity.

All of these methods are technically demanding and standardisation has not been achieved thus far. Studies using cut-off thresholds for CMV immunity are therefore not easily applied in routine clinical use. The only assay approved for clinical use for CMV immune monitoring is the QuantiFERON-CMV (Qiagen, Valencia, CA, USA), where whole blood is stimulated with CMV peptides and IFN-γ release is quantified by ELISA. This assay is analogous to the QuantiFERON-TB assay that has been widely adopted for tuberculosis. Although QuantiferON-CMV is less sensitive than flow cytometry assays, it is simple and reproducible and has been validated in HSCT recipients with similar results to other immune monitoring assays. There are no studies that yet conclusively demonstrate the safety of ceasing CMV monitoring based solely on the demonstration of CMV immunity. A future challenge is to integrate assessments of immune recovery into clinical algorithms of CMV management identifying patients in whom monitoring
can be reduced or eliminated and in whom pre-emptive antiviral therapy can be safely withheld.

**Interventions to improve CMV immune reconstitution**

Given the impact on mortality and cost, attention has focused on ways of improving CMV specific immunity as a means of improving overall outcome in transplant recipients.

**Vaccination**

The observation that antigenaemia is associated with improved CMV specific immune reconstitution implies that vaccination with antigen may improve immunity early post transplant at the time when patients are at risk of CMV. No CMV vaccine has regulatory approval for prevention of CMV infection in normal individuals but a number of vaccine candidates are under investigation. A recombinant glycoprotein B vaccine was trialled in seronegative and seropositive solid organ transplant recipients prior to transplant with significant improvement shown in antibody titres in both serogroups, along with a reduction in duration of viraemia and antiviral treatment required in D+/R− patients who reactivated post transplant. ASP0113 is a bivalent vaccine containing 2 plasmids that encode CMV glycoprotein B and tegument protein PP65. In a phase 2 randomized placebo trial of ASP0113 in which 40 allo HSCT CMV seropositive patients were administered the vaccine prior to conditioning and at 1, 3 and 6 mths post transplant there was a reduction in overall CMV viraemia, delay in viremic onset and reduced risk of recurrence. However there was no reduction in use of antiviral therapy and no significant improvement in measured CMV specific immunity. A planned HSCT donor vaccination arm for this study had to be abandoned due to logistical problems with identifying and vaccinating donors prior to transplant. This compound is now under investigation in a phase 3 study with planned recruitment of 500 participants (Clinicaltrials.gov ID NCT01877655). An alternative vaccine candidate is a peptide vaccine conjugated to a toll like receptor agonist that has been administered to 18 HLA-A201 CMV seropositive allogeneic stem cell transplant recipients at day 28 and day 56 as part of a randomized phase 1 clinical trial. A CMV-specific CD8+ T cell response was noted without an antibody response and both risk of CMV reactivation and duration of CMV antiviral therapy were reduced compared to the control arm. An increase in relapse-free survival was also demonstrated.

**Adaptive transfer of CMV specific T cells**

Infusion of unmanipulated donor lymphocytes after transplant can improve antiviral immunity but it does so at the cost of increased GVHD. CMV specific immune reconstitution via adoptive transfer of *ex vivo* isolated or expanded donor derived virus specific T cells (CMV-VSTs) has now been performed successfully in a number of clinical trials. CMV-VSTs can be manufactured in a variety of ways. Time consuming *ex vivo* culture methods using limiting dilution cloning or EBV transformed lymphocytes as antigen presenting cells (APCs) have largely been replaced with more rapid techniques using alternative APCs (activated monocytes, rapidly matured monocyte derived DCs or artificial APCs), various antigen sources and shorter culture times. *Ex vivo* culture duration can range from 10 to a number of weeks. Direct isolation methods using activation markers, cytokine capture or multimer selection are able to generate a clinical product within 48 hours. There are no studies comparing these methods directly, but it appears that cellular persistence depends on the presence of CD4+ cells either within the adoptive cell product or generated from the stem cell graft. Adoptive cell therapy appears safe, with no evidence that it increases the risk of GVHD in single arm studies and a cohort study. Two randomized studies of prophylactic/preemptive adoptive immunotherapy have been performed using direct capture methods for generation of donor-derived CMV VSTs (CMV~IMPACT and CMV~ASPECT) that have been reported in abstract form. Adoptive therapy appeared to be safe in both studies, and a significant expansion of CMV-specific cells was reported over controls for the ASPECT study. However, reductions in the rates of reactivation, recurrence and duration of therapy in the IMPACT study failed to reach significance, likely contributed to by a lower than expected CMV event rate in the control group.

Routine prophylaxis with transplant donor-derived CMV-VSTs is currently expensive, and may not be needed in patients who would never reactivate CMV. Thus, a pre-emptive strategy using rapidly generated CMV-VSTs may be a preferable approach although the methodology for cell generation is not yet widely available. An alternative approach is to use partially-HLA matched banked third party CMV-VSTs. To date, the treatment of over 75 patients with active CMV infection and disease utilizing banked non-donor derived unrelated (third party) CMV-VSTs have been reported. The 3 largest studies showed a high complete response rate, and did not flag any safety concerns; in particular the rate of GVHD was not higher than expected. In contrast to donor-derived adoptive transfer, these third party partially-matched donor cells do not appear to persist.
beyond a few weeks, yet effect long-term CMV control. With a median follow up of 6 months, few patients required retreatment with antiviral therapy after the final dose of third party cells.\(^{109,110}\) Further studies of third party cells are needed to elucidate the mechanisms of this clinical effect.

**Engineering of the graft to improve immune recovery**

As an alternative to the use of VSTs, graft engineering strategies may maintain antiviral immunity while reducing the risk of GVHD associated with transplantation of unmanipulated stem cell products. These include add-back of *ex vivo* alldedpleted T cells and naïve T cell depletion of stem cell products via CD45RA or TCRα/β depletion.\(^{111-113}\) Incorporation of safety switches into T cells prior to infusion allows selective deletion of gene-modified cells if GVHD occurs.\(^{114,115}\) Other immunomodulatory approaches to GVHD include induction of anergy or administration of regulatory T cells but the effect of these strategies on CMV immunity has not been studied.\(^{116,117}\)

**CMV immunity and AML relapse**

Donor seropositivity for CMV has been associated with lower relapse rate in AML in a number of observational studies dating back to the mid 1980s.\(^{118-123}\) CMV reactivation in patients undergoing transplant for AML was associated with reduced relapse risk with a proposed hypothesis of immune mediated anti-leukemic activity. In response to these studies the CIBMTR has performed an analysis of the impact of CMV reactivation on relapse in 9,469 patients with haematological malignancies, including 5,310 with AML.\(^9\) No association was found between CMV reactivation before day 100 and reduced risk of relapse. An association of CMV reactivation with reduced AML relapse risk in a transplant subgroup cannot be absolutely excluded. In contrast, CMV reactivation was associated with increased transplant related mortality and reduced overall survival.

**Conclusion and future directions**

With few exceptions, studies show that recovery of both CD4\(^+\) and CD8\(^+\) cellular immunity are required for protection from CMV viral replication. CMV seropositive recipients with donors who are seropositive for CMV have fewer, shorter and later viraeamic episodes compared with those with seronegative donors. Regular monitoring with pre-emptive antiviral therapy has reduced the rate of CMV disease and direct mortality but at a cost of morbidity and financial burden. Despite clear efficacy in minimizing CMV tissue infection, pre-emptive anti-CMV therapy has not eliminated the adverse effect of CMV reactivation on non-relapse and overall mortality and CMV remains a major contributor to poor post-transplant outcomes. It is not yet clear which patients with CMV reactivation can safely be left to clear virus without pharmacological intervention. The use of more specific measurements of immunity may facilitate better case selection for pharmacotherapy. Graft engineering strategies promise to preserve graft mediated anti-CMV activity but more rapid reconstitution of CMV immunity after transplant seems most likely to result from donor and/or recipient vaccination and adoptive immunotherapy.

**Abbreviations**

CMV = cytomegalovirus  
CMV-VSTs = CMV virus specific T cells  
GVHD = graft versus host disease  
HSCT = haemopoietic stem cell transplant

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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