Human cytomegalovirus (HCMV; also known as human herpesvirus 5) is the prototype member of the Betaherpesvirinae. Like all herpesviruses, it establishes latency and persists for the life of the individual. Infection with HCMV is common throughout the globe. The proportion of adults with specific IgG antibodies approximates to 60% in developed countries and more than 90% in many developing countries. Infection is more common in those from lower socio-economic groups and from non-Caucasian backgrounds. Children born in the UK to women who have moved from high-risk countries have the lowered risk of their adopted country. The saliva and urine of young children are major sources of virus, especially for those with childcaring responsibilities. HCMV is not highly contagious, with a basic reproductive number of ~1.7–2.4. It can also be spread sexually, by transfusion of whole blood or by organ transplantation. It is important to note that there are usually no symptoms associated with HCMV infection, except for occasional cases of infectious mononucleosis. This is because a robust immune response to HCMV normally prevents the high viral loads required to cause the end organ disease (EOD) seen in immunocompromised individuals. However, despite the absence of overt symptoms, there is evidence that infected individuals may have long-term adverse outcomes related to induction of a chronic inflammatory cell-mediated immune response to this apparently innocuous virus (indirect effects).

The natural history of HCMV infection is complex, with three different subtypes of infection. Primary infection occurs when an individual with no immunity against this virus becomes infected for the first time. Afterwards, the virus establishes latency from which it may reactivate (second type of infection). The third type of infection is called reinfection when contact with an infectious individual results in superinfection of someone who has already been infected, despite their possession of natural immunity. The third type of infection is called reinfection when contact with an infectious individual results in superinfection of someone who has already been infected, despite their possession of natural immunity. Any of these three subtypes of infection can complicate pregnancy, making HCMV the commonest cause of congenital infection. It is also the most common and the most serious opportunistic infection after solid organ transplantation (SOT) or haematopoietic stem cell transplantation (SCT) and remains an important opportunistic infection in individuals with HIV.

In this Review, we will focus on HCMV infection in immunocompromised individuals. We aim to integrate
information about serial measures of the viral load to explain features of pathogenesis and differences between distinct patient groups, show how active infection (although asymptomatic) is routinely monitored in selected patients and illustrate how prototype vaccines can be evaluated for efficacy. We will emphasize the evidence provided by double-blind, placebo-controlled randomized clinical trials (RCTs) of active or passive immunotherapy specific for HCMV.

HCMV immune evasion and viral latency

Our understanding of the complex interaction of HCMV with the immune response has been informed, in part, by comparative analyses of established laboratory-adapted strains of HCMV [BOX 1] and clinical isolates. An overview of infection and establishment of latency is shown in FIG. 1. Variation in sequence occurs naturally between HCMV strains and has the potential to impact pathogenesis and vaccine development. Note that individual genes of HCMV are numbered sequentially with the unique long, unique short or terminal repeat regions of the genome: thus, UL54 refers to the 54th gene in the unique long region.

Numerous studies have demonstrated that HCMV encodes countermeasures against a spectrum of immune responses14–16,18 (FIG. 2a). This arsenal of immunomodulatory functions is likely a reflection of the natural history of the virus, providing the capacity to establish lifelong infections of the host as well as to reinfect people with an existing infection despite the presence of a substantial immune response — particularly an enlarged T-cell compartment dominated by anti-HCMV T-cell responses that often exceed 10%17. The complexity of these immunological interactions has been reviewed extensively elsewhere18–20. Suffice it to say that HCMV-encoded gene functions target antigen presentation by major histocompatibility complex (MHC) class I and class II molecules, utilize cytokine mimicry to exert paracrine functions against immune cells and encode proteins that antagonize the range of innate immune responses directed against the virus (FIG. 2a). Despite this, HCMV infection or reactivation in the immunocompetent individual is rarely a cause of morbidity, implying that the surfeit of immune evasion mechanisms encoded by HCMV is imperative for long-term persistence in the host but not sufficient to completely evade immunosurveillance.

It is tempting to propose that the long-term solution for HCMV to evade immune responses is by ‘hiding’ from them, rather than ‘running’ from them, as seen in HIV infection21. This strategy of hiding from immunosurveillance is exemplified by the virus spreading cell to cell within a sanctuary site of persistence. This is coupled with an ability to establish latency, providing a mechanism by which the virus can go to ground if the immune system gains the upper hand, only to return later through reactivation if the immune system becomes impaired.

The ability to establish lifelong latent infections of the host is a defining characteristic of herpesvirus infections22. An overview of HCMV latency and reactivation is shown in FIG. 2b. HCMV establishes latent infections in bone marrow haematopoietic progenitor cells23. Additionally, tissue endothelial cells may be another reservoir of latent or persistent infection42. Latency can be defined as persistence of the virus, providing the capacity to establish lifelong infections of the host as well as to reinfect people with an existing infection despite the presence of a substantial immune response17. The complexity of these interactions has been reviewed extensively elsewhere18–20. Suffice it to say that HCMV-encoded gene functions target antigen presentation by major histocompatibility complex (MHC) class I and class II molecules, utilize cytokine mimicry to exert paracrine functions against immune cells and encode proteins that antagonize the range of innate immune responses directed against the virus (FIG. 2a).
The activity of inflammatory cytokines as inducers of MIE gene expression and HCMV reactivation (for example, tumour necrosis factor (TNF) and interleukin-6 (IL-6)) may be important in the process of organ transplantation which is associated with substantial inflammation. Indeed, one of the first reports of reactivation of naturally latent HCMV in vitro was achieved using the cocktail of cytokines released from allogeneically stimulated T cells. Furthermore, sepsis increases the incidence of HCMV reactivation in immunocompetent patients in intensive care, which could be linked with the inflammation associated with bacterial infection complications. Consequently, it is hypothesized that inflammation-associated signalling is a key driver of reactivation. The fact that HCMV has evolved mechanisms to modulate host cell signalling during latency and reactivation would be consistent with this.

A common refrain is that immunosuppression is a key trigger of HCMV reactivation yet HCMV reactivation can be modelled in vitro in cell culture systems. Thus, a distinction between clinical reactivation and cellular reactivation is important. Clinical reactivation is the detection of viraemia in individuals who are seropositive. Cellular reactivation is the re-initiation of viral replication in differentiated permissive cells. Importantly, understanding the relationship between these two events is likely crucial for understanding the basis of viraemia in immunocompromised individuals seropositive for HCMV.

Studies of cells isolated from healthy immunocompetent individuals have provided evidence of HCMV lytic gene expression in dendritic cells (cellular sites of HCMV reactivation) despite no evidence of viraemia. This observation suggests that HCMV latency and reactivation are an ongoing event in the host — an event that is controlled by the prodigious immune response directed against it. Indeed, this constant exposure of HCMV to the immune system likely explains the immunological space devoted to control of the virus. Thus, in individuals with compromised immune systems (for example, after organ transplantation), reactivation is still occurring but the loss of immune function allows the virus to replicate unchecked, leading to viraemia and, ultimately, disease. Importantly, in many of these scenarios where we observe immunosuppression there is a concomitant inflammatory state driven by co-infections or allogeneic T cell responses that exacerbate the situation due to the associated inflammation having proviral roles in replication.

It is noteworthy that HCMV is often one of the first viral pathogens to be identified diagnostically in patients with transplantation. This pre-eminent emergence is easily explained if we assume the virus is being controlled at the point of reactivation rather than at latency. Essentially, immunosuppression is akin to releasing the brake rather than representing the trigger. What remains to be understood is the relative contributions that different aspects of the innate and adaptive immune responses make towards the control of HCMV infection in vivo in different clinical settings of immunosuppression or immunodeficiency. The first clues will come from studies of immune function in different patient cohorts and, specifically, identifying the loss of which elements of the response are responsible for pathogenesis.

**Natural history and pathogenesis**

The natural history of HCMV consists of frequent infection that sometimes leads to viraemia. Only some of those with viraemia then proceed to develop EOD. In this section, we discuss each immunocompromised patient group in turn, each of which have different reasons for being immunocompromised. Notably, each EOD is similar across different patient groups (for example, HCMV retinitis has the same clinical features in all patient groups). Diagnosis of HCMV EOD requires evidence of symptoms at the affected site together with a biopsy showing histopathological changes of owl’s eye inclusion bodies and/or immunocyto logical staining to demonstrate productively infected cells, except in the case of retinitis where the characteristic clinical appearance of haemorrhage in retinal vessels accompanied by exudate is accepted.

Retinitis may be reported by the patient as a visual loss or ‘floaters’ passing across the visual field, or may be asymptomatic and identified by clinical examination alone. HCMV retinitis is attributed to lytic infection, a conclusion supported by clinical resolution with antiviral therapy (systemic ganciclovir plus intravitreal foscarnet if the retinitis is immediately sight-threatening). Healing is by fibrosis, predisposing the patient to future retinal detachment, a cause of major vision loss. When antiretroviral therapy was introduced, some individuals with HIV developed immune recovery uveitis, an inflammatory response to the presence of HCMV antigens within the eye. This condition may cause more visual disturbance to a patient than the underlying retinitis.

**Pneumonitis** is typically seen soon after SCT. The viral load in bronchoalveolar fluid is high. The pathology involves interstitial recruitment of lymphocytes increasing the distance that oxygen has to pass between the alveoli and blood vessels in the lung. There is evidence for an immunopathological contribution to this disease.

The remaining EODs of hepatitis, gastrointestinal ulceration and rarer conditions such as nephritis or pancreatitis are assumed to be caused by lytic infection.

**End organ disease in solid organ transplantation.** The very first cases of SOT were complicated by HCMV pneumonitis, with high mortality. The pioneers in organ transplantation mitigated this by lowering doses of immunosuppressive drugs, but at the risk of allowing graft rejection to occur. Balancing the need for immunosuppression (graft rejection) with a need for immune control (HCMV) benefited greatly from the development of less toxic immunosuppressive drugs coupled with the HCMV antiviral ganciclovir and its prodrug valganciclovir. However, the problem of HCMV EOD still persisted. Cases of HCMV pneumonitis continued to occur without prior warning: although many patients had fever, this sign is so common post transplant that it is not a specific indicator of HCMV infection. HCMV was also detected histopathologically in biopsies from other organs beyond the lungs, particularly the gastrointestinal.
**a**  
**Fibroblast entry**

- gM–gN
- gB
- gH–gL–gO

HSPG  
EFGR  
PDGFRα  
Integrins  
Cell-specific receptor

**Epithelial cell entry**

- gM–gN
- gB
- gH–gL–gO

HSPG  
EFGR  
PDGFRα  
Integrins  
Cell-specific receptor

**Non-permissive myeloid cell entry**

- gM–gN
- gB
- gH–gL–gO

HSPG  
EFGR  
Integrins  
Cell-specific receptor

- P13K
- c-Src

**Epithelial cell entry**

- gM–gN
- gB
- gH–gL–UL128–UL130–UL131

HSPG  
EFGR  
Integrins  
Cell-specific receptor

**Non-permissive myeloid cell entry**

- gM–gN
- gB
- gH–gL–gO

HSPG  
EFGR  
Integrins  
Cell-specific receptor

- P13K
- c-Src

**b**  
**pH-independent fusion (fibroblasts)**

- Capsid release

**Endocytosis (epithelial and endothelial cells)**

- Early endosome  
  - Capsid release

**Non-permissive myeloid cell entry**

- Early endosome  
  - Trans-golgi network  
    - Recycling endosome  
      - Capsid release

**c**  
**Capsid release**

**Cellular differentiation**

- CD34+ cells  
  - Activation signals

**Histone acetylation**

- Transcription factor binding to MIE

**Long-term silencing**

- Histones

**Transcriptional repression**

- ERF  
  - YY1

**Recruitment of repressive functions**

- Histones

- HDAC  
  - HMTS

**Transcriptional activation**

- IE genes

- MIEP

- Long-term transcriptional repression

- Long-term silencing
tract, in patients with oesophagitis, gastritis or colitis. It was also recognized in the eye when patients complained of visual disturbances (retinitis). These conditions were grouped together as ‘end organ diseases’ with a poor outcome.

Key to advancing the treatment of HCMV in SOT was defining the natural history of HCMV infection. Infection with this virus was then seen to precede EOD and to rise to high viral loads before EOD occurred. This gave an opportunity for active infection to be treated before it caused EOD; termed pre-emptive therapy (PET).

A series of studies in the 1990s demonstrated that the highest viral loads in urine from patients with renal transplantation were found in the donor positive–recipient negative (D+R–) combination; that is, the donor is infected with HCMV and the recipient has no natural immunity to this virus. Importantly, this subgroup also had the highest proportion meeting a case definition of HCMV EOD (signs and symptoms together with HCMV detected histopathologically in a biopsy of the affected organ). Importantly, multivariate statistical models were consistent with a high viral load causing EOD, rather than the alternative interpretation of the absence of natural immunity being responsible. Although elements of immune function are important, their contribution is captured by quantitative measures of viraemia.

**End organ disease in stem cell transplantation.** Evidence that viral load and natural immunity are an important component of EOD comes from studies of the other major transplantation cohort — individuals who have undergone SCT.

The first notable difference is that the high-risk group here is the D–R+ cohort (Fig. 3), and thus the opposite of individuals who have undergone SOT. This view is supported by the sero-epidemiology, which suggests that transmission from a D+ organ is rare in this setting. HCMV transmission is likely a function of the number of stem cells donated coupled with the very low frequency of latently infected cells (<0.01%) predicted to be in the graft. Additionally, in the D+ setting the graft also transfers HCMV cell-mediated immunity to the recipient. Thus, it follows that the adoptive transfer of HCMV immunity without a substantial increase in the risk of HCMV infection from a D+ individual leads to better outcomes than a D– graft into an R+ individual. Experimentally, immunization of donors prior to bone marrow harvest can transfer immunity to recipients.

Additionally, Fig. 3b shows that SCT patients have the same high risk of EOD as D+R– SOT patients despite the SCT patients being R+. By contrast, the two R+ SOT subgroups have a low risk of EOD (Fig. 3a). This shows that R+ SCT patients are more susceptible to EOD than R+ SOT patients, because there is a higher incidence and greater severity of EOD in this cohort. This is explained by SCT patients developing EOD at lower viral loads than SOT patients.
The advent of pre-emptive therapy to manage HCMV infection in transplantation. What became clear from studies of both SOT and SCT cohorts is that viraemia is a robust biomarker to predict individuals most at risk of HCMV disease post transplantation. In both transplant groups, the incidence of EOD was found to have a non-linear relationship with viral load, such that disease was uncommon until a high viral load was reached, with SCT patients being more susceptible to EOD than SOT patients. Importantly, all patients could be monitored and given antiviral drugs when a low threshold of viraemia was reached. Such PET is highly effective at preventing EOD (FIG. 5). An alternative strategy of giving antiviral drugs prophylactically is also effective, but at the risk of delaying EOD until prophylaxis is stopped (FIG. 5). Both strategies...
The presence of human cytomegalovirus (HCMV) within the bloodstream that is attached to white blood cells or within them are recommended in current clinical guidelines for the management of SOT and have served to reduce the number of individuals experiencing EOD\textsuperscript{45,61}. In a recent RCT, patients with liver transplantation randomized to management with PET had significantly less late-onset EOD than those managed with prophylaxis therapy\textsuperscript{62}. The concept that PET allows low-level antigen presentation to the immune system is supported by studies of humoral and cell-mediated immunity post transplantation\textsuperscript{63}. In other words, the low levels of viraemia that occur in individuals monitored by PET have a low risk of causing EOD yet are sufficient to stimulate the immune system to bring viraemia under control.

These quantitative studies characterized a series of parameters that can be used to define the severity of HCMV (proportion of individuals with viraemia, duration of viraemia and peak viral load). These parameters are now sufficiently robust to be accepted by regulators as end points for RCTs\textsuperscript{64}. Importantly, these surrogate markers of EOD also allow the continued study of HCMV natural history and pathogenesis without compromising patient treatment. For example, they revealed that the replication dynamics of primary HCMV infection in vivo is very similar to that of HIV infection — and much quicker than anticipated based on studies of the development of cytopathic effects in in vitro culture models\textsuperscript{43,44}.

All of these observations were assimilated into a dynamic model of HCMV infection and EOD. Within hours of transplantation, HCMV reactivates from the donor organ. This productive infection may be controlled by the local immune response (FIG. 2). If it is not, HCMV appears in the blood, allowing the virus to disseminate to multiple organs. Note that viraemia can be detected as a leukoviraemia or a plasma viraemia. Plasma viraemia (strictly plasma DNAemia) consists of short, fragmented portions of HCMV naked DNA within blood\textsuperscript{65}.

Although it is clearly not infectious, plasma viraemia still acts as a good biomarker for taking decisions about PET\textsuperscript{44,45}. The physical state of the virus is not defined in the case of leukoviraemia. Separation techniques using magnetic beads followed by quantitative PCR for HCMV DNA revealed that polymorphonuclear leukocytes make the largest contribution to the overall viral load in blood, and that HCMV DNA and late mRNA transcripts can be found in monocytes, B cells and T cells, consistent with productive infection\textsuperscript{66}. The response to ganciclovir was similar when HCMV DNA was measured in each of these cellular fractions of peripheral blood\textsuperscript{67}. Viraemia is not a guarantee of EOD because immune responses (FIG. 2) at the level of each organ may be able to prevent blood–organ transmission of virus and/or the development of EOD. If these immune responses are insufficient, HCMV may rise to high levels, causing EOD through various potential pathological processes. For example, low levels of virus may not complete a full replicative cycle (FIG. 1) yet display HCMV antigens on cells to make them targets for immunopathological responses.

There is some evidence for such responses in the lungs\textsuperscript{41}, although a high viral load is also found in bronchoalveolar lavage fluid in individuals with extensive, established pneumonitis\textsuperscript{42}. Higher levels of virus may lead to productive infection (FIG. 1) with lysis of target cells; the retina would be a potential site for this, although it may also be followed by immune recovery uveitis, which is an immunopathological condition\textsuperscript{42}. In all cases, initial immunosuppression, which may involve steroids that are given to all SOT patients, has an effect by increasing the viral load in the blood\textsuperscript{64}. By contrast, steroids given to treat graft rejections in SOT (or graft versus host disease in SCT) increase the risk of EOD by lowering the viral load required to cause disease (that is, steroids are statistically independent from a high viral load as a risk factor for EOD in multivariate models)\textsuperscript{47}.

In summary, there is evidence for both viral lysis and immunopathology contributing to EOD. However, invasive samples from the affected site are only available from the late stages of disease when early immunopathological responses may have been joined by lytic destruction of target cells producing a high viral load. 
**End organ disease in individuals with HIV.** HCMV retinitis presented as a major complication in the dawn of the AIDS epidemic, with EOD most likely to occur in individuals seropositive for HCMV (and thus a result of HCMV reactivation or infection)\(^7\). Indeed, it is a startling clinical observation that retinitis accounts for 85% of EOD in individuals with HIV compared with only 1% of individuals in the transplant groups; with no proven explanation for this. A possible explanation is that damage to the blood–retina barrier due to HIV infection may facilitate HCMV gaining preferential access to that organ.

A major difference between these individuals and the transplant cohorts is the absence of a starting point equivalent to the date of transplant to indicate when the risk of HCMV EOD increases. The major indicator is when the CD4\(^+\) T cell count of individuals with HIV falls below 100 cells per microlitre of blood. In these individuals, natural history studies showed (FIG. 4a) that HCMV becomes detectable and rises to high levels in the blood, similar to those found in D+R– SOT patients\(^8\).

Thereafter, individuals are at risk of developing EOD, but the temporal dynamics are altered; instead of preceding EOD by weeks (SOT) or days (SCT), individuals with HIV can have high HCMV viral loads for months before developing EOD\(^9\). One possible explanation we can offer is that blood–organ barriers (apart from the retina) are better preserved in individuals with HIV than in the transplant groups.

**The ‘indirect effects’ of HCMV infection.** EOD associated with HCMV has been well described in numerous important patient populations. What is less clear are the associated ‘indirect effects’ of HCMV infection and replication. This term was coined by Rubin to describe the unexpected high prevalence of conditions such as accelerated atherosclerosis seen in cohort studies of patients with heart transplantation with active HCMV infection\(^7\). This condition was not unique to HCMV infection, but the virus increased its incidence. Potential mechanisms that could lead HCMV to contribute to atherosclerosis include systemic inflammation, monocyte activation, T cell stimulation and effects on the endothelium\(^7\).

The evidence for HCMV causing such phenomena comes from observations made in subjects enrolled in double-blind, placebo-controlled RCTs. For example, accelerated atherosclerosis was significantly reduced by prophylaxis with ganciclovir in patients with D+R– heart transplantation\(^7\). Likewise, the incidence of biopsy-confirmed acute graft rejections after renal transplantation was significantly reduced in an RCT of high-dose valaciclovir in the D+R– subgroup, but not in the recipients who are seropositive\(^7\). It is often said that the low levels of viraemia found in patients managed by PET must increase their risk of graft rejection. In fact, a meta-analysis by the Cochrane collaboration of the RCTs conducted to compare PET with prophylaxis show no differences for graft rejection, graft survival or patient survival\(^7\).

In the SCT population, high mortality linked to HCMV serostatus is observed, even in the absence of overt HCMV-driven EOD\(^7,\)\(^\#\). Death in SCT patients is divided into relapse-related (that is, recurrence of leukae­mia) or transplant-related (for example, opportunistic infections). Recipients of transplants who are seropositive for HCMV have an increased transplant-related mortality that was reduced by acyclovir prophylaxis\(^7,\)\(^8\). The interpretation of these observations was limited by the broad-acting nature of acyclovir, but subsequent studies with HCMV-specific letermovir significantly reduced mortality as a predefined secondary end point of the RCT\(^7,\)\(^8\). Importantly, it was the ability of letermovir to prevent viraemia that conferred the statistical benefit of reduced mortality\(^7,\)\(^8\).

As with SCT, the major indirect effect of HCMV infection in individuals with HIV is death not explained by EOD\(^7,\)\(^8\). Interestingly, it is HCMV viraemia and CD4\(^+\) T cell count, and not HIV loads, that are the correlates of mortality\(^7,\)\(^8\). Consistent with this, systemic
exposure to ganciclovir in individuals experiencing their first episode of retinitis reduced mortality rates\(^4\). A meta-analysis of RCTs of acyclovir also showed a significant reduction in mortality\(^5\).

**Mechanisms by which HCMV has been shown to interact with HIV in vitro are transactivation of HIV gene expression and pseudo-type formation, both of which require the two viruses to infect a single cell\(^6\). Four other mechanisms that require the two viruses to infect neighbouring cells are stimulation of cytokine release, antigen presentation, upregulation of CD4 or its co-receptor and induction of an alternative entry receptor for HIV\(^7\). The plausibility of these interactions occurring in vivo was supported by detecting the nucleic acid of both viruses in more than 50% of tissues sampled at autopsy\(^8\). However, no evidence was found to support the prediction that HIV loads increase in individuals who are HIV-positive co-infected with HCMV (reviewed in ref\(^9\)). Attention therefore moved to an alternative mechanism based on the induction by HCMV of an excess of immunocommitted CD8\(^+\) T cells as part of its contribution to the ‘immune risk phenotype’ or ’immunosenescence’ that is associated with increased prevalence of atherosclerosis in individuals with HIV\(^10,11\). The possibility of a causal relationship was supported by the observation that the level of HCMV-specific CD8\(^+\) T cells was decreased significantly in a small RCT of valganciclovir\(^12\).

Overall, these observations are strikingly similar to those in the SOT and SCT populations, but have been largely overlooked by the HIV research community despite similar results being reported every few years\(^13\). We continue to suggest that studies of both HIV and transplantation could potentially benefit from collaboration to explore and compare potential mechanisms; for example, the observed increase in the prevalence of atherosclerosis after SOT could be explained by the excess of inflammatory T cells that has been reduced in an RCT involving individuals with HIV. This is important because the total amount of morbidity caused by the indirect effects of HCMV may exceed that currently attributed to EOD\(^14\). Furthermore, clinicians should be aware that ‘silent’ HCMV infection may be predisposing various patients to adverse outcomes, including excess mortality in the general population, increased duration of ventilation when patients are admitted for intensive care following heart attacks, burns or sepsis and increased severity of COVID-19 (refs\(^15\)–\(^18\)). The important principle is that underlying HCMV infection induces a long-term inflammatory bias that can contribute to other medical conditions without manifestation of its presence.

**Immune correlates of HCMV control**

What is clear from our understanding of clinical HCMV infection is that pathogenesis is mainly observed in individuals with poor immune responses. That said, the precise component of the immune response responsible for protection is still unclear. For example, active HCMV infection is seen in individuals with poor cell-mediated immunity measured against the MIE antigen or pp65 proteins — two immunodominant antigens\(^19\). However, it has been difficult to define cut-off levels at baseline or at the end of prophylaxis to identify which SOT patients are not at risk of infection\(^20\). This is partly because of the fluctuating risk seen with time, as some patients require additional immunosuppression in the form of
It is also partly because clinicians wish to be informed preferentially about the highest-risk patients, yet these are the D+R– subgroup where measurements of specific immunity in the recipient are undetectable. Furthermore, studies of recipients who are seropositive often fail to differentiate between control of reactivation or reinfection. Recently, a single paper has produced substantial evidence focused on the D+R+ subgroup that immune responses to the MIE antigen detectable pre transplantation predict the risk of HCMV viraemia post transplantation.

One potential issue with current strategies is that focus has often centred on measuring the quantity rather than the quality of the immune response. This may not simply be a numbers game — it may be more a question of having the right response rather than a large response. A pivotal study demonstrated that the T cell response against HCMV is diverse and targeted against the full range of HCMV proteins. These observations have been substantiated in numerous smaller, more focused studies that essentially demonstrate that the response is dynamic and broad. Ongoing studies are addressing whether infusion of HCMV-specific T cells into patients can provide protection.

Antibodies, natural killer cells and macrophages may theoretically contribute to a protective immune response and are expected to interact and cooperate with T cells to control HCMV replication. A very recent paper has reported some of the immune functions that require collaborative contributions from more than one component of the immune system by studying viral proteins expressed at the surface of the infected cell and determining which could mediate antibody-dependent cellular cytotoxicity. Remarkably, these targets were not the major structural glycoproteins of the virus but the proteins it deploys as immune evasins. Future studies of this kind have the potential to give a more sophisticated assessment of the immune capability of individual patients at risk of HCMV infection.

**Emerging strategies to treat infection**

Ganciclovir was licensed in 1989 and remains the only licensed drug potent enough to treat active HCMV infection. Although the oral prodrug valganciclovir was licensed in 2001, it delivers the same active ingredient. For strains of HCMV resistant to ganciclovir, foscarnet is used off-label. Clearly, this field would benefit from more licensed drugs that are both safe and effective against HCMV. One important outcome of comprehensive studies of the natural history and pathogenesis of HCMV is the provision of strong evidence that measuring the viral load is a robust surrogate for measuring EOD, and thus a requirement for PET. This becomes particularly important for clinical trials seeking to test the anti-HCMV activity of novel compounds.

Three phase II RCTs have been conducted in the context of SCT using PET as the read-out to determine whether novel antiviral drugs given prophylactically can control HCMV viraemia better than placebo. The first drug was maribavir, which reduces the ability of the virus to break down the nuclear membrane required to allow newly formed virions to escape from the nucleus of an infected cell. The second drug was brincidofovir, which, similar to ganciclovir, inhibits the virus-encoded DNA polymerase. The third drug was letermovir, which inhibits the terminase complex that takes newly synthesized HCMV DNA in concatameric form, cuts it into genome lengths and packages these into nascent virions. All three drugs were successful without causing bone marrow suppression and proceeded to phase III studies.

In the first phase III study (maribavir), EOD was required as the primary end point. The drug failed to reduce EOD for two reasons: PET was allowed for
patients in both arms of the study and rescued those who had failed prophylaxis; and the sponsors chose the lowest dose of drug instead of the highest non-toxic dose. In the second study (brincidofovir), PET was allowed as the primary end point, but a drug-free washout period was included after the end of prophylaxis. The drug initially suppressed the need for PET but this difference then declined with time, leaving no overall significant difference when compared with placebo. The reason for this was an excess of graft versus host disease in the drug arm of the study that was treated with steroids that helped protect recipients who are seropositive from HCMV viraemia. Many of these clinically diagnosed cases were not true graft versus host disease (which is classically diagnosed with diarrhoea, rash and abnormal liver function) but simply cases of diarrhoea caused as a known side effect of brincidofovir. The third drug (letermovir) reduced PET significantly and was licensed for use. While these RCTs were in progress, regulators in the USA and the EU progressively accepted that EOD was an undesirable and impractical end point and that the need for PET was now appropriate for phase III studies. Studies of future drugs should therefore now be more straightforward to conduct. Two other aspects of regulatory requirements for phase III studies now also need to be brought up to date. First, drugs for prophylaxis should be given immediately post transplantation rather than waiting for engraftment, which is a hangover from studying the bone marrow toxic drug ganciclovir. Second, there is no scientific rationale for requiring a washout period after prophylaxis ends before assessing whether the need for PET has been reduced. This is not a requirement for anti-HIV drugs and is another hangover from the original ganciclovir study. Thus, application of modern understanding of the natural history and pathogenesis of HCMV is rapidly improving clinical trial design.

**Evaluation of novel vaccines**

There is no doubt that the development of vaccines to protect against HCMV infection or disease will be complex. This virus can establish lifelong latency and immune individuals can experience repeat infections from endogenous (reactivation) or exogenous (reinfection) sources despite the host committing substantial immune resources against HCMV. An early RCT gave live attenuated Towne vaccine strain (BOX 1) or placebo to candidates who are seronegative awaiting renal transplantation. Post transplantation, the incidence of HCMV infection and EOD was not reduced but the severity of EOD was. The subsequent development of quantitative PCR allowed the viral load parameters described above to be used as pharmacodynamic read-outs to determine whether vaccines have activity against HCMV replication in these patient populations.

A vaccine consisting of viral glycoprotein B (gB) with MF59 adjuvant given prior to SOT showed reduced post-transplantation viral load parameters when compared with recipients of placebo. The correlate of immunity protection was the titre of IgG antibodies against HCMV. Antibodies against the immunodominant antigenic domain 1 were not protective, consistent with the possibility that the presence of this domain represents another example of HCMV evading protective immune responses. These hypotheses should be tested formally in future RCTs.

To test that antibodies were a mechanistic correlate of protection, Genentech evaluated placebo-controlled infusion of preformed monoclonal antibodies specific for HCMV at the time of D+R– renal transplantation. The company conducted an RCT in 120 individuals and demonstrated significant interruption of transmission of HCMV from donor to recipient. This approach of using active and passive immunization serially and in tandem in SOT should be applied to the evaluation of novel vaccines in the future.

Disappointing results were recently presented orally with a DNA plasmid vaccine in SOT that appeared to be poorly immunogenic and did not reduce the need for PET. When these phase III study results are published, it will be important to determine whether the change from immunizing donors in the encouraging phase II study was important.

Two more HCMV vaccines have proceeded to phase II studies. Hookipa Pharma presented a modified lymphocytic choriomeningitis virus construct to
express either gB or pp65. Co-administration of both constructs produced good humoral and cell-mediated responses and the results of a phase II study in patients who are seronegative with renal transplantation are awaited14. Positive results from this study could lead to RCTs in women of childbearing age at risk of primary infection. Merck have engineered two proteins within HCMV strain Ad169 modified to express the penta-meric complex by fusing two viral proteins (IE1/2 and pUL51) to the destabilizing domain of FK506-binding protein 12. This fusion targets these essential proteins for degradation by the proteasome unless an exogenous chemical is present15. The resulting genetically inactivated whole virus strain is being studied in women of childbearing age who are seronegative but could easily be applied to immunocompromised individuals in the future. When the results of these two studies are published, it will be possible to review the evidence for reduced primary infection, examine the immune correlates of protection and make recommendations for whether either or both products should proceed to phase III studies. These studies will be larger versions of the current phase II studies, with at least 30,000 women who are seronegative required. The primary end point will also change from primary infection in the women to congenital infection in their neonates. We recommend that such studies in women and the SOT population should proceed in parallel because of the similarities of HCMV in both patient populations16. Meanwhile, the same and/or different vaccines should be studied for their ability to ‘boost’ or ‘improve’ the natural immune response to HCMV so that the incidence of reactivations or reinfections can be reduced. The SOT population routinely monitored by PCR and managed by PET represents an ideal population to study. We also recommend that studies of active immunization should proceed concurrently with studies of passive immunotherapy using monoclonal antibodies with defined reactivity against specific proteins of HCMV; the SOT population acts effectively as a human challenge model to facilitate such studies.

Conclusions and open questions

We have reviewed how a virus that does not declare its presence by producing specific symptoms can nevertheless be monitored prospectively to define quantitative parameters of replication. These measures can be deployed for PET to reduce EOD and to define immune correlates of control. By giving a prototype vaccine or placebo pre transplantation, the viral load parameters can be used as pharmacodynamic read-outs of successful protection. Passive transfer of monoclonal antibodies or T cells can then be used to both confirm the immune correlate and establish a medically acceptable new treatment. Clinical cohorts continue to report reduced survival of patients with grafts and/or allografts in subgroups at risk of active HCMV infection12,15, so the goal should be to return these parameters to the values found in the D–R– subgroup. Although HCMV represents a complex target, we are optimistic that serial rounds of iterative studies will finally bring this important and under-recognized human pathogen under control.

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**Author contributions**

Both authors researched data for the article. P.G. wrote the first draft, which was revised by M.R. Both authors contributed substantially to the discussion of content, reviewed the text and edited to form the final manuscript.

**Competing Interests**

Both authors are co-inventors (along with I. Baraniak) on UK patent application number 2020135.6 assigned to University College London (UCL), entitled “hCMV antibody and vaccine target”, that deals with a novel antigenic domain on HCMV glycoprotein B (gB). UCL received funds from Takeda pharmaceuticals to compensate for the time P.G. spent as a member of the end-point committee for a randomized clinical trial (RCT) of maribavir. The authors declare no other competing interests.

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