Recent Advances in Cytomegalovirus: An Update on Pharmacologic and Cellular Therapies

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Abstract

The 2015 Tandem American Society for Blood and Marrow Transplantation/Center for International Blood and Marrow Transplant Meetings provide an opportunity to review the current status and future perspectives on therapy for cytomegalovirus (CMV) infection in the setting of hematopoietic stem cell transplantation (HSCT). After many years during which we have seen few tangible advances in terms of new antiviral drugs, we are now experiencing an exciting period of late-stage drug development, characterized by a series of phase III trials incorporating a variety of novel agents. These trials have the potential to shift our current standard therapeutic strategies, which generally involve pre-emptive therapy based on sensitive molecular surveillance, towards the prophylactic approaches we see more generally with other herpes viruses such as herpes simplex and varicella zoster. This comes at a time when the promise of extensive preclinical research has been translated into encouraging clinical responses with several cellular immunotherapy strategies, which have also been moved towards definitive late-stage clinical trials. How these approaches will be integrated with the new wave of antiviral drugs remains open to conjecture. Although most of the focus of these cellular immunotherapy studies has been on adaptive immunity, and in particular T cells, an increasing awareness of the possible role of other cellular subsets in controlling CMV infection has developed. In particular, the role of natural killer (NK) cells is being revisited, along with that of T cells. Depletion of NK cells in mice results in higher titers of murine CMV in tissues and increased mortality, whereas NK cell deficiency in humans has been linked to severe CMV disease. We will review recent progress in these areas.

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Over the past 2 decades, both prophylaxis and preemptive therapy have been used to prevent CMV disease in the HSCT setting [1]. Although preemptive therapy is most commonly used, prophylaxis is favored by some centers for high-risk patients, such as recipients of unrelated, HLA-mismatched or cord blood products. Although both strategies are effective for prevention of CMV, they rely on available drugs with significant toxicities, including marrow toxicity for ganciclovir, valganciclovir, and cidofovir, and renal toxicity for foscarnet and cidofovir [2,3].

The treatment of CMV disease after HSCT typically consists of ganciclovir at induction doses for 2 to 3 weeks, followed by maintenance dosing until all signs and symptoms are undetectable. When cytopenias are present, foscarnet is used as alternative. Valganciclovir is sometimes used after an initial response is documented, provided that there is good oral intake and adherence to the regimen; however, no systematic evaluations of this approach exists. Although CMV gastrointestinal disease can be treated with an antiviral drug alone, recommendations for CMV pneumonia include the addition of intravenous immunoglobulin [2,4]. Drug-resistant CMV disease is rare after HSCT but should be suspected in patients with poor clinical or virologic responses and pre-exposure to the antiviral drug used. Patients who are on antiviral drugs and who have had viral load increases for more than 2 weeks may have resistance. If drug resistance is suspected, genotypic testing and switching to an alternative drug is recommended as first-line approach [2,5]. Viral load can be used to monitor the response to treatment. In patients with documented drug resistance or those who are critically ill, often few options exist and none are
supported by high-quality data. Novel agents described below may be available for use in some situations.

**Future Approaches**

Because of the safety profile of currently available drugs, efforts have been made in developing new compounds with similar or improved efficacy and improved toxicity. Also, several presently available drugs have been reported to have anti-CMV activity in vitro. The following summarizes new antiviral agents being evaluated in clinical trials in HSCT recipients.

Maribavir, a UL97 protein kinase inhibitor, is an oral drug with specific activity against CMV [6]. A phase II dose-ranging study in HSCT recipients showed that CMV infection or disease was reduced at all 3 dose levels tested, but a subsequent phase III study that used the lowest dose (100 mg twice daily) failed to prevent CMV disease [7]. The failure of the study was primarily attributed to the dose used in that study [8]. Maribavir has in vitro activity against ganciclovir- or cidofovir-resistant CMV, and small case series suggest a possible clinical benefit at higher doses [9]. Therefore, 2 ongoing phase II dose-ranging trials are examining higher doses of maribavir treatment of refractory or resistant CMV disease (clinicaltrials.gov NCT01611974) and as preemptive therapy (EudraCT: 2010-024247-32).

Letermovir (AIC-246), a CMV terminase inhibitor, is another highly selective anti-CMV agent [10,11]. The drug can be given orally or intravenously and is highly active against wild-type and drug-resistant CMV in vitro. In vivo experience for multidrug-resistant CMV disease is limited [12]. A phase II dose-escalation study in CMV-seropositive HLA-matched HSCT recipients showed a reduction of prophylaxis failure (defined as drug discontinuation due to CMV infection or disease or any cause) in patients receiving the 240 mg of letermovir compared with those receiving placebo [13]. The drug was tolerated well, with similar adverse event rates in letermovir and placebo recipients. A phase III randomized multicenter trial is currently ongoing using a similar trial design as the phase II trial (clinicaltrials.gov NCT02137772).

Brincidofovir (CMX-001) is a new broad spectrum antiviral agent that has in vitro activity against herpesviruses, polymaviruses, adenoviruses, papillomaviruses, and variola virus [6]. It is a lipid-conjugated nucleotide analogue of cidofovir that has a high oral bioavailability and long half-life, allowing twice-weekly oral dosing. In contrast to its parent compound, brincidofovir is not a substrate for the human organic anion transporters and, therefore, has significantly reduced potential to cause renal toxicity. A phase II dose-escalation study in HSCT recipients showed a reduction of CMV infection or disease in patients receiving brincidofovir at doses of 200 mg per week for prophylaxis started at engraftment [14]. The most common side effect was diarrhea in patients receiving CMX001 at doses of 200 mg weekly or higher. It was dose limiting at 200 mg twice weekly. There was no difference in renal or hematologic adverse effects between brincidofovir and placebo recipients. A phase III randomized multicenter trial of brincidofovir at a dose of 100 mg twice weekly is currently ongoing using a similar trial design as the phase II trial (clinicaltrials.gov NCT01769170).

Leflunomide is a Food and Drug Administration—approved drug for the treatment of arthritis with documented activity against several viruses, including CMV and BK virus [15]. Leflunomide has been used in salvage situations for CMV disease with mixed results [16]; however, no systematic evaluation of the efficacy and toxicity of leflunomide either as mono- or combination therapy has been performed.

Finally, artesunate is an antimalarial agent that also has broad antiviral activity in vitro against herpes viruses [17], hepatitis viruses, and human immunodeficiency virus because of its ability to downregulate NF-κB or Sp1 pathways [18]. There are anecdotal reports of its effectiveness in patients with complicated CMV infection, including multidrug-resistant CMV [19]; however, no systematic evaluation of the efficacy and toxicity of artesunate for CMV treatment has been performed.

**Future Perspectives**

Preemptive antiviral therapy substantially reduced the incidence of CMV disease after HSCT in the past 20 years. Several new drugs are now in advanced stage of clinical evaluation and may be available for more effective and less toxic prevention of CMV in HSCT recipients. Studies are also needed to determine whether these drugs can be used in combination to reduce mortality of CMV pneumonia.

**ADVANCES IN T CELL THERAPIES**

Because the primary risk factor for CMV infection after HSCT is considered to be a deficit in number and function of CMV-reactive T cells [20], a number of investigators have addressed the possibility that adoptive transfer of donor-derived (and, in some cases, third-party) CMV-reactive T cells will hasten reconstitution of protective pathogen—specific immunity, potentially reducing the infective burden and associated treatment costs [21]. Derivation of a therapeutic cellular product is technically easiest when the original stem cell graft donor has pre-existing immunity to CMV. In these cases, direct selection of virus-specific T cells, or expansion of such cells in ex vivo, is usually feasible. Most of the early demonstrations of proof of concept relied on an ex vivo expansion step, limiting more widespread clinical application [22,23]. Subsequent refinements in culture conditions allowed more rapid cell expansion [24-27]. More recently, increasingly robust strategies for direct selection of virus-specific T cells from seropositive donors have been developed, including selection after restimulation with viral peptides according to secretion of IFN-gamma or up-regulation of cell surface activation markers [28,29], or direct selection of unstimulated cells based on binding of class I HLA-multimers [30,31]. Each strategy produces a therapeutic product that differs in terms of cellular composition, purity, antigen specificity, and functional characteristics. Application in subsequent phase I and II studies has also introduced further variation in terms of the cell doses employed, and the timing of and indication for intervention (eg, prophylactic, preemptive, or for clinically “resistant” infection). Nevertheless, most clinical studies reach a broadly similar conclusion: immunity can be restored in the absence of significant toxicity and with a low risk of induction of graft-versus-host disease (GVHD) [32]. Of course, early phase studies may be influenced by selection biases, and exclusion of those with clinically significant active GVHD is an obvious bias of these early studies. Furthermore, there are data to suggest that immune reconstitution after HSCT is dependent to some degree on the frequency of CMV-specific T cells in the donor graft. Because low precursor frequency correlates with failure to generate a therapeutic product in some cases, a further bias is introduced in uncontrolled studies. These
considerations highlight the fact that cellular therapies need to be evaluated just like any other drug in prospective randomized confirmatory studies.

The design of confirmatory studies employing cellular therapeutics, particularly if delivered without the financial support of large pharmaceutical industry partners, has to take into consideration a number of key factors relating to the incidence of CMV infection and likelihood of generation of a patient-specific bespoke product that is not then utilized because of lack of infection or precluding clinical features, such as GVHD. For these reasons, we focused on CMV-seropositive recipients receiving T cell—depleted grafts. This patient group has a very high incidence of CMV infection and a low baseline incidence of GVHD. The development of "direct" selection technologies that obviate the need for ex vivo expansion also aids trial development. Two randomized confirmatory studies have recently been completed in the United Kingdom; 1 a study of prophylaxis in the sibling donor setting (IMPACT) and the other of preemptive therapy in the unrelated donor setting (ASPECT), using CMV-specific T cells selected either by HLA-streptamers (IMPACT/ASPECT), or by gamma-catch technology (IMPACT) according to HLA type. The control groups received standard viral PCR-based surveillance, with standardized criteria for intervention and stoppage of antiviral drugs. Further studies of CMV-specific T cell therapies are planned in the T cell—replete setting and in patients receiving corticosteroids for GVHD. The results will require integration with changes in practice that evolve with the availability of new antiviral drug therapies. For example, it is well known that acyclovir is very effective at preventing varicella infection/reactivation, but also that a significant fraction of patients will experience symptomatic infection when prophylaxis is stopped because of a lack of reconstitution of protective immunity while receiving antiviral therapy [33]. A similar picture has been reported with the use of prophylactic ganciclovir for CMV, with delays in restoration of protective immunity presumably relating to lack of exposure to viral replication and DNAemia during prophylaxis [34]. It is possible that the combination of adoptive cell therapy and an abbreviated period of prophylaxis with a new antiviral drug will prove optimal.

There are limitations associated with some of the new strategies. The HLA coverage of a given population will vary widely according to geographical location and local variations. Thus, therapies targeting populations with specific HLA expression patterns will be more or less relevant according to the nature of local patient and donor populations. CMV seropositivity rates are also variable. There are a number of potential solutions being evaluated for patients without seropositive donors. A "third-party" bank of virus-specific cells offers a number of potential advantages in terms of models of delivery and opportunities for commercialization. Rapid availability for use directed by a "best available HLA match" algorithm (wherein the transferred cells need to recognize the pathogen in the context of a shared HLA allele) is particularly attractive. Although proof-of-concept is available [35], similar issues regarding possible selection bias need to be addressed in larger confirmatory studies before more widespread adoption. Potential issues here relate to possible alloreactivity in either a host-versus-graft direction, resulting in rejection of the adoptively transferred populations before they can exert the desired effect, or in the opposite graft-versus-host direction, resulting in third-party GVHD if the cells engraft robustly. Whether third-party cells engender more rapid reconstitution of second-party immunity derived from the original stem cell donor, either by acting as a cellular vaccine or through a brief burst of lysis of virally infected host cells, remains unclear. If such strategies were proven to be clinically effective, they would likely be more easily applied on a widespread basis than the alternative strategy of induction of primary immune responses ex vivo, with subsequent expansion and adoptive transfer [36].

Alternative strategies are also being evaluated that may achieve a broader repertoire of immune reconstitution against both known and unknown pathogens. These include transfer of memory T cell populations depleted of the naive compartment that contains most of the alloreactive potential of the graft [37]. Delivery in the absence of GVHD would offer significant advantages over more piecemeal reconstitution to multiple known pathogens.

ADVANCES IN UNDERSTANDING THE BIOLOGICAL ROLE OF NK CELLS

NK cells were first described as a function in the 1960s. Lethally irradiated and unsensitized mice were capable of rejecting bone marrow cell allografts. Later, this hematopoietic stem cell (HSC) resistance was found to be different than classical solid tissue allograft rejection in that F1 hybrid mice were capable of rejecting parental bone marrow cell allografts, placing this phenomenon at variance with the classical laws of transplantation mediated by T cells and solid tissue allografts [38]. This phenomenon was called hybrid resistance. It was in 1975 that this in vivo phenomenon could be tied with the ability of peripheral blood mononuclear cells to spontaneously lyse transformed or virally infected cells in a non-MHC restricted manner [39]. The cell type responsible for these effects was found to be a large granular lymphocyte that shared many but not all markers present on T cells. The definition of an NK cell arises, therefore, by its function (usually spontaneous non-MHC restricted lysis of a tumor cell line, such as K562 in human or YAC-1 in mice) and phenotype, which is characterized by the presence of some markers (eg, CD56 in humans, NK1.1 in mice, and CD16 as well as CD16 in both) and absence of others (eg, CD3 and TCR). This inclusion-exclusion phenotypic approach is important, as many of the markers used to identify NK cells can also be expressed on other cell types (eg, NKT cells also express NK1.1). The ability to mediate antibody-dependent cell-mediated cytotoxicity is another important distinguishing activity mediated by NK cells. As more and more of the biology of these cells has been gleaned over the years, it is clear that they are far more complex than originally thought and they comprise different subsets with a very unique receptor system that regulates their innate ability to detect and attack targets. It is the emergence of these subsets that indicate NK cells exert important functions outside of cellular cytotoxicity.

NK Cell Biology

The original studies on NK cells and their biology were performed using inbred mice. Early studies indicated that these cells were critically dependent on the bone marrow microenvironment for development and differentiation as marrow ablation resulted in loss of in vivo function [40]. Indeed, NK cells were at 1 point called “M cells” because of this dependence on the marrow, which made them distinct from other hematopoietically derived lymphoid cells (ie, T and B cells), which could develop by extramedullary hematopoiesis [40]. NK cells predominantly circulate in the
hematopoietic system (peripheral blood, spleen, liver), but they can also be found in other tissues, particularly after an inflammatory response to infection. Numerous cytokines play a role in NK cell development and/or function. Initially, IL2 was used to demonstrate activating and proliferative effects on NK cells, but it was later found that although this cytokine was sufficient to induce these responses, it was not obligatory, as IL2 knockout mice displayed normal NK cell function [41]. The critical importance of IL15 for NK cell development and homeostasis was demonstrated by the absence of NK cells in IL15 and IL15R knockout mice, and by the inability of these mice to support the survival of adoptively transferred mature NK cells [42]. There are multiple stages in NK cell development that precede exportation into the circulation and functional maturation. Pivotal on these is acquisition of MHC-binding receptors. These receptors allow the NK cell to “see” and respond to virally infected and transformed cells. Herein also is a significant divergence between mouse and man. Human NK cells express the killer cell immunoglobulin-like receptor (KIR), which binds to HLA class I molecules. Mouse NK cells express Ly49 molecules, which are structurally distinct but functionally similar to KIRs. Both of these receptors recognize MHC class I molecules and have ITIM (inhibitory) or ITAM (activating) signaling motifs, which dramatically affect NK cell activity. Adding to these are other receptor systems (NKG2A and D, 2B4), which also have ITIM and ITAM motifs and affect function. They are not exclusive and NK cells bear many of these receptors in differing amounts, which affects overall net function on NK when it binds to a target. These receptors can also be modulated depending on the environment [43], which also affects overall responses. It is the existence of these different yet similar receptor systems that indicates NK cells have developed to be tightly controlled. Although they represent an important arm of the innate immune response, protection from auto-reactive responses mediated by NK cells is pivotal. There are also other cell types and cytokines that regulate NK cell function and development. Regulatory T cells have been demonstrated to directly inhibit NK cell activity in vitro and suppress their ability to reject allogeneic HSCs in vivo [44]. Transforming growth factor–beta is a cytokine that also suppresses NK cell function, although it can also be produced by activated NK cells [45]. It is the understanding of agents that activate NK cells as well as pathways that inhibit their function that has drawn much interest with regard to clinical applications in cancer.

**NK Cell Modeling**

To understand NK cell biology and potential clinical applications, it is imperative to have an appropriate model. As the initial studies characterizing NK cells were done using inbred mice, it is perhaps not surprising that the majority of subsequent studies and assessment of potential clinical applications have also been performed in mice. However, as more has been gleaned from human studies either by in vitro assessment or by observing effects after clinical trials, it is very clear that there are significant differences between the species that need to be taken into consideration before extrapolating mouse data to humans.

First and foremost, there are significant genetic species differences illustrated by the different receptor systems (ie, KIR in humans and Ly49 in mice), absence of CD56 on mouse NK cells and the presence of CD56 subsets (eg, CD56hi) in the human lymph node, whereas NK cells are lacking in mouse lymph nodes unless stimulated. The presence of these CD56hi NK cell subsets within the human lymph node indicate that NK cells likely play a role in other critical functions, such as immune homeostasis and immune regulation. CD56lo NK cells in peripheral blood are highly lytic, whereas the CD56hi subset secretes cytokines but is poorly lytic [46]. There are also differences in the ability to culture NK cells long term, as mouse NK cells invariably die after several weeks in culture despite optimal cytokines, whereas human NK cells are able to be cultured for much greater periods of time and even cloned [40,47]. Another large variable is the simple fact that inbred mice are housed under specific pathogen-free conditions and this can markedly affect NK cell function. Regardless, the similarities between the species still far outweigh the differences and simply need to be kept under consideration.

Classically, NK cells have been demonstrated to mediate direct antitumor and antiviral immunity. First, they have shown to be capable of mediating direct killing of the aforementioned cell types. NK cells kill by perforin/granzyme, which results in direct lysis of the cell and can be assessed by short-term assays. NK cells also kill by fas ligand and TNF-related apoptosis-inducing ligand (TRAIL) [48], and this can be observed using longer term in vitro assays. Finally, NK cells can also secrete effector cytokines, such as interferon-gamma and TNF [49]. All of these pathways likely work in concert in vivo and contribute to the net function of the NK cell on a pathogen. Recently, it has been elegantly demonstrated that NK cells also exert potent immunoregulatory functions on adaptive immune responses during viral infection. It was observed that NK cells could directly inhibit T cell-mediated responses and memory to viral infections [50]. This study indicated that the NK cell was capable of directly killing the activated T cells, resulting in lesser pathology during the viral infection. Interestingly, it was also recently demonstrated that NK cells and T cells could directly compete with each other with regard to their effector functions [51] and numbers, indicating that a complex pattern exists affecting the overall immune response. This in part could be due to direct (ie, killing of activated T cells via NKG2D ligand recognition) and indirect (ie, competition for cytokines, such as IL15) mechanisms. Additionally, NK cells have been demonstrated to modulate immune responses by both promoting and killing dendritic cells [52], it is likely the overall or net immunoregulatory function of NK cells is contingent on the location and extent of the inflammatory response.

NK cells classically have been demonstrated to mediate resistance to viral infections, although certain viruses (eg, CMV) have developed means to avoid NK cell attack by pirating MHC-like domains and, thus, directly inhibiting NK responses [53]. In mice, murine CMV resistance has also been shown to be affected by NK cells expressing the Ly49H–activating receptor (in C57BL/6 mice), which has an ITAM and leads to the expansion and activation of this subset in response to infection [54]. Additionally, as NK cells are the first lymphoid cell to repopulate after HSCT, studies in mice have shown even greater roles for them after HSCT and subsequent infection. Furthermore, it has been reported that as opposed to what is classically thought to be an innate cell type, the expanded Ly49H+ NK cells exhibit “memory” responses upon reinfection, thus blurring the lines between NK and T cells [55]. The role of other NK subsets in these responses has not been clarified but it is likely that subsets with different ITIM receptors play differential roles, based on the observation that these subsets undergo different developmental responses due to
licensing" or "arming." It has been reported that the NK cell subsets bearing different Ly49 receptors in mice are capable of binding self MHC to varying degrees. Those capable of strongly binding self MHC have been reported to exhibit an increased ability to produce interferon-gamma in response to stimulation in vitro versus those NK cells bearing Ly49 or NKG2A receptors with ITIM that bind weakly [56]. The physiological role of licensing during viral infections is still unclear, but as with T cell subsets (ie, Th1, Th2, Th17, regulatory T cells), differential effects will likely be observed. The observation that the different subsets in mice had different biologic effects was first reported using allogeneic HSCT and looking at engraftment as a readout [40]. It was observed that the MHC-binding capability of the different Ly49 members could affect whether resistance occurred. When a host NK cell came across an allogeneic HSC not bearing "self" MHC to turn off the NK cell, the HSC would then be attacked, resulting in graft rejection. It was in this manner that the different Ly-49 subsets could be shown to play a specific role in HSC rejection contingent on the MHC haplotype of the recipient. It was also demonstrated that NK cells could mediate resistance to any allogeneic hematopoietically derived cell, including lymphocytes, as well as preferentially target transformed cells of hematopoietic origin. In mouse HSCT models, it was also demonstrated that NK cells of donor origin could suppress GVHD and promote graft-versus-tumor effects in part through their production of TGF–beta [57]. This was then extended with preclinical and clinical studies indicating that NK cell subsets could indeed influence outcome after allogeneic HSCT based on Ly49 (mouse) or KIR (human) expression and haplotype.

CONCLUSIONS

Since its description in neonates in the early 20th century as the cytomegalic inclusion disease, characterized by an often fatal systemic infection associated with the detection of large cells in the urine, CMV has remained something of an enigma. During recent decades, advances in diagnostic tests and treatments have improved our ability to manage CMV infection in immunocompromised hosts, but the virus still accounts for significant morbidity and mortality. New antiviral drugs, and advances in our ability to utilize virus-specific T cells to hasten immune reconstitution will likely provide further patient benefits in coming years. Further dissection of the roles of both innate and adaptive immunity will also play a role. It is important to remember that the current delicate balance that exists between pathogen and host, which seems so well adapted as to maintain an almost symbiotic relationship, is relatively easily perturbed with potentially catastrophic consequences, and that our understanding of the full spectrum of risks and benefits of CMV infection and its interactions with the host immune system remains far from complete.

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