Human cytomegalovirus and transplantation: drug development and regulatory issues

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Abstract

Cytomegalovirus (CMV) infection is highly prevalent worldwide and can cause serious disease among immunocompromised individuals, including persons with HIV and transplant recipients on immunosuppressive therapies. It can also result in congenital cytomegalovirus when women are infected during pregnancy. Treatment and prevention of CMV in solid organ and haematopoietic stem cell transplant recipients is accomplished in one of three ways: (1) prophylactic therapy to prevent CMV viraemia; (2) pre-emptive therapy for those with low levels of replicating virus; and (3) treatment for established disease. Despite the high prevalence of CMV, there are few available approved drug therapies, and those that are available are hampered by toxicity and less-than-optimal efficacy. New therapies are being developed and tested; however, inconsistency in standardisation of virus levels and questions about potential endpoints in clinical trials present regulatory hurdles that must be addressed. This review covers the current state of CMV therapy, drugs currently under investigation, and clinical trial issues and questions that are in need of resolution.

Keywords: Cytomegalovirus, CMV, regulatory science, drug development

Introduction

Cytomegalovirus (CMV), a large opportunistic double-stranded DNA virus in the *Herpesviridae* family [1], infects approximately 60% of individuals in developed countries and nearly 100% of individuals in developing countries [2]. Although the majority of infections are asymptomatic, morbidity and mortality is high for immunocompromised individuals, and congenitally infected infants [1,3]. The three main types of infection are: primary, reinfection and reactivation. Primary occurs in patients with no pre-existing immunity, after which CMV establishes latency and viraemia is controlled mainly by cell-mediated immunity [1]. Reinfection occurs in patients with insufficient natural immunity to prevent a subsequent external infection, whereas reactivation occurs in individuals whose natural immunity is insufficient to protect against endogenous infection.

In transplant patients, CMV in the bloodstream (DNAemia) invades the organ system to cause end-organ disease. The indirect effects of CMV on the immune system lead to increased risk of additional infections and promote graft rejection [1]. Transplant recipients with primary infection are most at risk for severe morbidity, and available strategies to avoid CMV disease include the use of prophylactic or pre-emptive therapy. Prophylactic therapy is initiated at the time of the organ transplant or stem cell engraftment, whereas pre-emptive therapy is initiated in high-risk asymptomatic patients when diagnosed with primary CMV infection, when they reach a pre-defined threshold of CMV DNAemia [1]. Pre-emptive treatment, now standard-of-care, has significantly reduced CMV disease in immunocompromised transplant patients [4].

CMV retinitis was a major disease in HIV patients, resulting from reactivation of latent virus or reinfection. As treatments became available, this manifestation became less common in developing countries, but remains of concern [1]. In addition, CMV acts as an inflammation activator and is associated with inflammation-dependent co-morbidities in HIV patients [5].

Another major population at risk for CMV-related sequelae are congenitally infected infants. CMV damages more babies globally than Down's syndrome, spina bifida, congenital rubella, *Haemophilus influenzae* and HIV combined [4]. CMV infects babies *in utero*, with an estimated 1-in-150 babies born in the US with CMV [3]. Congenital CMV may be asymptomatic or can result in birth defects and even death [3]. Approximately 15% of infants with congenital CMV infection have permanent disabilities including hearing loss, microcephaly, intracranial calcifications, ventriculomegaly, and other mental deficits such as psychomotor and perceptual disabilities [1].

The high burden of CMV disease and morbidity makes it a good candidate for new therapies and vaccines. Current treatments for CMV include antiviral therapies such as ganciclovir, its prodrug valganciclovir, and foscarnet. Current therapies are inadequate because of severe toxicities as well as an emergence of single- and multidrug-resistant strains [6]. That this is an instance of unmet medical need is clear, with urgent need for consistency in approach to drug development and access at the global level.

This review focuses on drug development and regulatory issues in the transplant setting. In a field which has not seen new drugs come to market for over 20 years, regulatory agencies and sponsors need to catch up with advances in the clinical and diagnostic setting. While these advances have been significant, the field has not benefited from the level of standardisation witnessed in HIV and HCV, where quantitative diagnostics, drug development, regulatory guidance and clinical standard-of-care co-evolved in real time.

Current drugs for CMV

Any new drug will need to be compared to placebo and then the current standard-of-care for prophylaxis and pre-emptive therapy in haematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients, primarily ganciclovir, foscarnet and, in rare instances, cidofovir (See Table 1).

Ganciclovir

Ganciclovir and its valyl prodrug valganciclovir have been the first-line therapy in management of CMV for the last two decades [7]. Ganciclovir, first approved in 1989 as an intravenous formulation for the treatment of CMV retinitis in AIDS patients, was later approved for treatment of CMV in SOT patients and for prevention of CMV in patients with advanced HIV. Oral ganciclovir has been available since 1994, but has poor bioavailability (5%)

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Table 1. Comparison of use and issues between current and in development CMV therapies. Adapted and expanded from [8]

Current drugs			
Ganciclovir (GCV)	Oral	Limited use as maintenance, or for mild/localised disease	Risk of myelosuppression; low bioavailability (5%); cross resistance; effective for CMV treatment, no longer preferred
	Intravenous infusion	Treatment, antiviral prophylaxis, and pre-emptive therapy in transplant recipients; CMV retinitis in HIV patients; non-retinitis CMV disease in HIV patients	Risk of myelosuppression
	Intraocular Implant	Treatment of CMV retinitis in combination with systemic GCV or VGC	
Valganciclovir (VGC)	Oral	Treatment, antiviral prophylaxis, and pre-emptive therapy in transplant recipients; CMV retinitis in HIV patients; non-retinitis CMV disease in HIV patients; May be used for induction or maintenance	Risk of myelosuppression; hallucination and neurotoxic effects in high doses; preferred for pre-emptive CMV in SOT and HSCT recipients
Foscarnet (FOS)	Intravenous infusion, intravitreal injection	Treatment, antiviral prophylaxis, and pre-emptive therapy in transplant recipients; CMV retinitis in HIV patients; non-retinitis CMV disease in HIV patients	Risk of nephrotoxicity; cross resistance; second-line therapy
Cidofovir (CDV)	Intravenous infusion	Treatment of CMV disease in transplant recipients; Treatment of CMV retinitis; Treatment of non-retinitis CMV disease in HIV patients	Risk of nephrotoxicity; cross resistance; second-line therapy
Drugs in development			
Maribavir (MBV)	Oral	In Phase 2 developments for SOT and HSCT	Taste disturbance over 800 mg/day; cross resistance may be possible
Brincidofovir (BCV)	Oral	In Phase 3 developments for SOT and HSCT	GI toxicity (diarrhoea) cross resistance may be possible; intravenous formulation in preclinical development
Letermovir (LMV)	Oral	In Phase 3 developments for SOT and HSCT	No indication of nephrotoxicity or haematological toxicity; no cross resistance due to unique mechanism of action.

and is no longer produced. A ganciclovir sustained-release intraocular implant was approved in 1996 for the treatment of CMV retinitis [1]. Valganciclovir, approved in 2000, replaced oral ganciclovir in practice because of its higher absorption, with a bioavailability of 55–60% [1,8].

Ganciclovir is an acyclic guanosine analogue activated via a multistep triphosphorylation process catalysed by the UL97-encoded viral kinase followed by cellular kinases [1,8]. Ganciclovir interrupts synthesis of viral DNA via competitive incorporation, eventually leading to DNA chain termination [1,8]. This mechanism of action contributes to ganciclovir resistance via UL97 gene mutations, and less commonly, mutations in the CMV DNA polymerase-encoding UL54 gene [1,8].

Ganciclovir is associated with significant bone marrow toxicity, resulting in neutropenia, thrombocytopenia and anaemia [1,8]. These toxicities contribute to the need for additional treatment options. In cases of prolonged and low-dose therapy, there is concern for the selection of ganciclovir resistance [1].

Foscarnet

Foscarnet, approved in 1991, was the second drug approved in the US for treatment of CMV retinitis [1]. Foscarnet is a second-line therapy generally reserved for patients failing ganciclovir due to drug resistance [8]. It may also be administered to patients with neutropenia, where ganciclovir is contraindicated [1,8].

Foscarnet sodium is a pyrophosphate analogue that binds to the pyrophosphate-binding site, blocking cleavage of pyrophosphonate from the terminal triphosphate added to the growing DNA chain and therefore inhibiting viral DNA polymerase [1]. Resistance to foscarnet is associated with point mutations in the UL54 gene, which encodes for polymerase [1,8]. Cross-resistance between ganciclovir and foscarnet has been identified in both clinical and laboratory isolates [1].

The most serious clinical side effect is renal toxicity [1,8] resulting in electrolyte imbalance and potentially resulting in death.

Cidofovir

In 1996, cidofovir was approved for treatment of CMV retinitis in AIDS patients. With low oral bioavailability (<5%), the drug is only available as an intravenous formulation [1]. Cidofovir is stable in its active form within cells, distinguishing it from other drugs in its class [8]. This is considered a second-line therapy and is generally reserved as an alternative for treatment of resistant strains [1,3,9,10].

Cidofovir is an acyclic nucleoside phosphonate broad-spectrum antiviral [1,8]. It has potency against many viruses within the *Herpesviridae* family as well as other DNA viruses [8]. Cidofovir diphosphate is a competitive inhibitor of viral DNA polymerase, resulting in early chain termination during DNA synthesis [1,8]. Accumulation of cidofovir in the renal cortex causes severe renal toxicity [8]. Resistance has not been reported, but treatment periods are generally shorter, which may prevent development of detectable resistance.

Alterations to cidofovir that may eliminate toxicity are being explored. One study looked at esterification of cidofovir in order to increase bioavailability, as well as decrease renal toxicity via reduction in accumulation of the drug in the kidneys [11]. A lipid prodrug is in Phase 3 development (see brincidofovir below).

Aciclovir

Aciclovir, in the form of high-dose oral valaciclovir has documented efficacy as antiviral prophylaxis to prevent CMV in kidney transplant recipients [8].

Fomivirsen

An intraocular injection of fomivirsen, an anti-sense RNA specifically targeting mRNA of an early CMV transcriptional unit

was approved in 1998 as a second-line therapy for the treatment of CMV retinitis in AIDS patients [12] but has been discontinued in the US.

Drugs in clinical development

The drug development histories of new CMV drugs illustrate the complexity of the disease (each transplant patient is really a donor–recipient system) and the need for an alternative to CMV disease as an accepted endpoint for drug approval. The FDA still requires clinical endpoints, but given the advances in CMV treatment, even with imperfect drugs, the incidence of clinical endpoints has been reduced significantly, making trials that depend on them a challenge.

Maribavir

Maribavir prevents the exit of new CMV virions from the nucleus by inhibiting UL97-mediated phosphorylation of nuclear Lamin A/C [13]. This is normally mediated by CDK1 in uninfected cells during mitosis. Resistance is associated with mutations in UL97 and has been observed in clinical and laboratory settings [13].

Phase 1 clinical trials demonstrated no adverse effects of concern, other than taste disturbance associated with doses higher than 800 mg/day [13]. Thus clinical trials used doses of 800 mg/day and then lower doses in subsequent trials [13]. Earlier Phase 2 and Phase 3 trials were unsuccessful in demonstrating efficacy based on primary endpoints [14].

The design issues with maribavir development have been discussed in detail elsewhere [13,15]. The Phase 3 study failed to demonstrate a statistically significant decrease of incidence of CMV infection measured via antigenaemia [14]. There was speculation that the choice of dose, exclusion of high-risk patients, and highly sensitive PCR paired with low CMV disease in the control group were the major contributing factors to this failure [13]. Moreover, the trial design did not take advantage of maribavir's lack of bone marrow toxicity, which would have allowed initiation of treatment before engraftment. Overall, the experience amply illustrates the difficulty of depending on prevention of CMV disease as an endpoint at a time when surveillance and pre-emptive treatment standard-of-care have advanced to the point that disease incidence is (fortunately) very low. CMV disease occurred at a rate of 4.8% – implying that required sample sizes for future trials would be prohibitive for many drug developers [14].

Open-label studies indicate that maribavir has an antiviral effect. The drug has been picked up by Shire Pharmaceuticals Ltd. Under the new development programme, two Phase 2 treatment studies with maribavir have been successfully completed. The first study was a randomised, dose-ranging, Phase 2 study in SOT and HSCT recipients resistant or refractory to prior anti-CMV treatment. The second study was a Phase 2 dose-ranging study, maribavir versus valganciclovir for pre-emptive treatment of asymptomatic CMV infection in HSCT and SOT recipients. Currently, two Phase 3 treatment studies are being planned (Shire, personal communication). We await the publication of their findings with anticipation.

Brincidofovir

Brincidofovir is cidofovir with a lipid side chain, which mitigates the severe cidofovir-associated renal toxicity. It is well absorbed intracellularly, where the lipid chain is cleaved [7]. The active drug is thus less likely to continue circulating, so reducing a toxic effect on the renal tubules [16]. It is associated with a dose-limiting toxicity to the gastrointestinal (GI) tract, but does not have detectable levels of toxicity in bone marrow [16].

Brincidofovir is currently being developed by Chimerix and the development programme included three pivotal trials [17]. The first was a safety and efficacy study to assess the effectiveness of brincidofovir over placebo in preventing CMV infection in previously uninfected HSCT recipients (referred to as SUPRESS) [11]. The second, SUSTAIN, was a randomised multicentre Phase 3 study to compare efficacy of brincidofovir versus valganciclovir in preventing CMV disease in kidney allograft recipients [18]. The third, SURPASS, was a randomised multicentre study of efficacy, safety and tolerability in brincidofovir- versus valganciclovir-treated kidney transplant recipients [19].

SUPRESS aimed to elucidate whether brincidofovir could prevent clinically significant CMV disease in HSCT transplant patients through 24 weeks post-transplantation [17]. At 14 weeks, SUPRESS did show a difference in incidence of CMV disease between brincidofovir and control patients (24% *vs* 38%, respectively), but there was no difference among control and treated groups at 24 weeks (52% *vs* 51%, respectively) [20]. Again illustrating the complexity of CMV disease in already immunosuppressed patients receiving HSCT from various donor types, several factors were implicated in the findings, including the higher use (eight times higher compared to placebo arm patients) of corticosteroids and other immunosuppressive interventions for the treatment of *presumptive* graft versus host disease. One of the side effects of brincidofovir is diarrhoea, which should have been managed with temporary treatment interruption rather than immunosuppression. In some cases, it appears that diarrhoea triggered a clilnical diagnosis of graft-versus-host disease and immunosuppression instead. In light of the SUPRESS results, the sponsor elected to close the SUSTAIN and SURPASS trials. At this point, the formulation of an intravenous therapy is progressing towards clinical trials, which may circumvent adverse GI effects [20]. Chimerix is committed to continue to explore brincidofovir as prophylaxis for CMV in HCT recipients, as an approved drug for this is still not available [20].

Letermovir

Letermovir has a unique mechanism of action. It is a terminase inhibitor, the same class of drug as tomelglovir (BAY38-4766) and GW275175X, two drugs previously studied as potential anti-CMV therapies, which were never pursued into clinical development [21]. Letermovir interacts with the pUL56 viral terminase subunit complex [13], preventing cleavage of long DNA concatamers leading to production of non-infectious particles [21]. This mechanism of action is not associated with cellular toxicity, a current unmet need in CMV therapeutics [21,22]. An additional benefit is the lack of cross-resistance with other anti-CMV therapies, including other terminase inhibitors [21].

Letermovir was licensed to Merck, which is conducting a Phase 3 trial to evaluate oral letermovir for CMV prophylactic activity in transplant patients. This is a randomised, interventional, efficacy study to assess clinically significant CMV up to 24 weeks [23]. Letermovir currently has 'orphan drug' status; the monitoring of clinical resistance will be important as more studies are conducted [21]. A dose-range finding prophylaxis trial that assessed different doses showed efficacy and little toxicity in 240 mg/day doses, and low efficacy with insufficient viral suppression of reactivation and replication as well as treatment-emergent letermovir resistance in 60 mg/day doses. The 240 mg/day dose may decrease the likelihood of resistance because it is efficacious for complete suppression of CMV viraemia [22].

Other relevant work

Cyclopropavir, with a mechanism of action similar to ganciclovir, is in Phase 1 safety studies by the National Institute of Allergy and Infectious Diseases. Promising data suggest that cyclopropavir and its derivatives may have stronger antiviral efficacy than cidofovir against herpesviruses including CMV [24]. Additionally, CMV-specific cytotoxic T lymphocyte (CTL) infusions are being assessed for effect on viral load as well as reconstitution of antiviral immunity [25].

Regulatory pathways for CMV drug approval

The FDA provides two pathways for approval (Table 2; Figure 1). The first, traditional (or regular) approval requires a clinical endpoint (how a patient feels, functions or survives) or a surrogate endpoint *known* to predict clinical benefit on irreversible morbidity or mortality. The second, the Accelerated Approval Pathway (21 CFR 314.510 and 601.41 Subpart H and E) is available for therapeutics intended to treat serious and life-threatening disease *and* that provide a meaningful benefit over existing therapies. This pathway requires demonstration of efficacy based on a surrogate endpoint that is 'reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence to predict benefit'. The distinction in use of a surrogate marker for traditional versus accelerated approval lies in the interpretation of 'known to predict' versus 'reasonably likely to predict'. Not unexpectedly, the field may lack consensus on when the evidence suffices for full approval. This lack of consensus may manifest itself in different requirements by different regulatory agencies.

Table 2. CMV DNAemia and drug development in 2016

- Regulatory pathways
  - Traditional or full approval in US and Europe requires demonstration of clinical benefit (clinical endpoint)
  - Accelerated (or conditional) approval is based on a surrogate endpoint 'reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence to predict clinical benefit'
    - Advantage of surrogate endpoint: allows smaller and shorter trials
  - Therapeutics approved through the accelerated approval mechanism may be marketed at time of approval
    - Require follow up trials demonstrating clinical benefit for full approval
  - Once a surrogate marker is validated and accepted as 'known to predict clinical benefit on irreversible morbidity or mortality' it may replace the clinical endpoint
- Regulatory status
  - In the US, CMV DNAemia is accepted as a surrogate endpoint for accelerated approval
  - In Europe, CMV DNAemia is accepted as a true endpoint for full approval
- Implications for future research
  - Need to demonstrate convincingly that CMV DNAemia levels correlate with disease
  - Need to demonstrate convincingly that reductions (or increases) in CMV DNAemia correlate with reduction (or increase) in risk of disease
- Challenges with implementation and interpretation of quantitative CMV DNAemia assays
  - CMV DNA testing on whole blood can yield results 10–100 times higher than on plasma, but can occasionally yield lower results
  - There were no FDA approved assays before 2012 and 'in-house' assays for CMV DNA show poor inter-laboratory correlation
  - WHO international reference standard should reduce variability between assays
  - In a 2015 report, up to 50% of 10 different real-time quantitative PCR assays run by eight different labs showed poor commutability

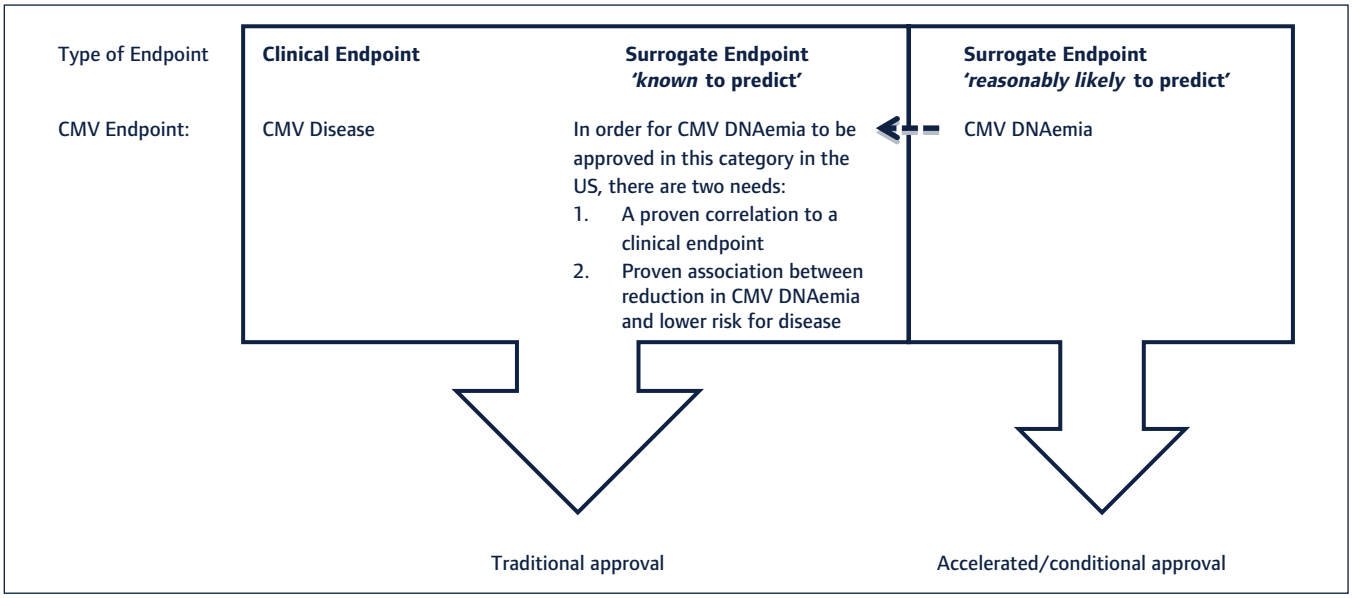

Figure 1. Regulatory path of CMV DNAemia as an endpoint in clinical trials

Surrogate endpoints are beneficial for drug development, particularly for chronic diseases where a validated biomarker can be measured earlier than a clinical endpoint. One major benefit of surrogate endpoints is the ability to conduct smaller, shorter clinical trials. Additionally, surrogate biomarkers are well suited for initial pharmacokinetic–pharmacodynamic assessments, dose findings, and making go/no-go decisions. All of these are important considerations for efficient drug development.

Validating a biomarker as an endpoint for clinical trials has been accomplished for other viral diseases Most famously, HIV/AIDS ushered in the concept of accelerated approval; HIV RNA was the first surrogate marker to be approved, first for accelerated approval, later for traditional approval [26]. In HCV, sustained virological suppression is accepted as a true endpoint for development of direct-acting antivirals. Many argue that CMV DNAemia should already be accepted as a surrogate endpoint for CMV trials based on substantial natural history data [4,27–30].

In the US, CMV DNAemia is accepted to be 'reasonably likely to predict' clinical outcome [16]. To turn CMV DNAemia to 'known to predict' clinical outcome, it needs to be convincingly correlated with a clinical endpoint and must fully capture the net effect of the intervention [26]. In other words, the field needs to demonstrate that: (1) CMV DNAemia correlates with disease; and (2) that reduction in CMV DNAemia is associated with lower risk for disease.

In Europe, regulators are more open to accepting CMV DNAemia as a surrogate endpoint for demonstrating antiviral efficacy. Relevant clinical outcomes would still be collected, but not needed as clinical endpoints for formal efficacy demonstration. Adapting trial results from one transplantation setting to another would require an appropriate treatment strategy (dosing, duration, need for combination therapy) and understanding of how setting and patient characteristics impact the desired clinical benefit [31].

Independent of its level of acceptance as a surrogate, CMV DNAemia tests face other challenges. Quantitative CMV DNA testing performed on whole blood can yield results 10–100-times higher than tests performed on plasma; yet occasionally, CMV DNA measurements in plasma may also be higher than in whole blood [27]. Prior to 2012, there were no FDA-approved assays for CMV DNA and inter-laboratory correlation for viral load values was poor. The COBAS AmpliPrep/COBAS TaqMan is approved, and has been calibrated against the now available WHO international reference standard [32]; demonstrating high inter-laboratory agreement and precision [33]. A WHO international reference standard [32] should reduce variability between assays. However, it remains to be seen to what extent 'in-house' PCR assays will provide consistently reliable and uniform values [28]. Recently, Hayden and colleagues reported poor commutability, with up to 50% of assays using 10 different real-time quantitative PCR assays run by eight different laboratories [34]. Clearly more work remains to be done to bring the field up to consistent and reliable performance.

Discussion

CMV is associated with more disease, morbidity and mortality than is currently acknowledged. Lack of acknowledgement, along with the complexity of disease in patient populations, have contributed to very slow progress toward finding better treatments. For the last few decades, the only antiviral therapies for use against CMV infection in all patient populations have been associated with severe toxicities. In addition, the use of antiviral therapies currently on the market has led to single- and multidrug-resistant CMV. Drug development is currently focused on CMV as it is related to SOT and HSCT patients, but the need for effective treatment goes beyond this type of patient. Treatment of CMV can extend to patients who have inflammation-dependent co-morbidities, complications associated with congenital or perinatal CMV infection, and those who have symptomatic primary infection, including pregnant women. New classes of therapeutics should also reduce the development of resistance and will allow for treatment of CMV without the need to balance risk of toxicity. The field as a whole needs to find ways to collaborate across stakeholder groups to increase the efficiency of drug development for this area of great unmet medical need [29].

Better and safer therapeutic options would facilitate the introduction of universal surveillance, diagnosis and screening, and treatment to prevent the significant morbidity and mortality associated with this very common yet largely unrecognised virus [35]. HIV and HCV have amply demonstrated that effective therapies incentivise screening, diagnosis and access to care. The massive attention given recently to viruses such as Zika may pave the way for higher incentives for treatment and prevention of known congenital viral infections.

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