Antiviral Therapies for Herpesviruses: Current Agents and New Directions

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ABSTRACT

Purpose: The objective of this review was to summarize the recent literature describing the current burden of disease due to herpesviruses in the antiviral and transplant era; describe mechanisms of action of antiviral agents and the development of resistance; summarize the literature of recent antiviral agents brought to market as well as agents under development; and to present literature on future strategies for herpesvirus therapeutics.

Methods: An extensive search of the medical literature related to antiherpesviral therapy was conducted to compose this narrative review. Literature searches were performed via PubMed and ultimately 137 articles were included as most relevant to the scope of this article.

Findings: Herpesviruses are a family of DNA viruses that are ubiquitous throughout human populations and share the feature of establishing lifelong infections in a latent phase with the potential of periodic reactivation. With the exception of herpes simplex virus, varicella zoster virus, and Epstein-Barr virus, which have a significant disease burden in individuals with normal immune function, the morbidity and mortality of the remaining viruses are primarily associated with the immunocompromised host. Over the last half-century, several agents have been tested in large randomized, placebo-controlled trials that have resulted in safe and effective antiviral agents for the treatment of many of these infections.

Implications: With increasing use of antiherpesviral agents for extended periods, particularly in immunocompromised hosts, the emergence of resistant viruses has necessitated the development of newer agents with novel targets and better side-effect profiles. (*Clin Ther.* 2018;40:1282–1298) © 2018 Published by Elsevier Inc.

Key Words: antiviral resistance, antiviral therapy, herpes simplex virus, cytomegalovirus, varicella zoster virus.

INTRODUCTION

The human herpesviruses (*Herpesviridae*) are a large family of DNA viruses that occur throughout human populations and establish lifelong latency subsequent to primary infection.[1] Clinical manifestations and diseases due to these infections vary widely. One commonality among these viruses is that reactivation of viral replication in profoundly immunocompromised individuals is possible and can be associated with significant morbidity and mortality.[2–4] It is primarily in this patient population that the need exists to develop more potent and safer antiviral agents as the incidence of antiviral resistance continues to increase.[5–8] The evidence regarding genetic diversity among clinical isolates of these viruses suggests that a combination approach of different viral targets should be explored to mitigate the emergence of resistance mutations and treatment failures; a similar method has been successfully shown with the treatment approach for HIV infection.[9,10] Therapies that can target the latent phase of these viral infections could potentially result in eradication, while ongoing efforts to develop vaccines for certain herpesviruses could mirror the success achieved with varicella zoster virus (VZV) vaccines.

The present review briefly outlines the burden of disease due to herpesviruses and the growing problem of antiviral resistance. Current and newly approved therapies are reviewed, including mechanism of action and resistance mechanisms. Finally, antiviral therapies in development are presented, including stage of development.

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BURDEN OF DISEASE

The herpesvirus family is divided into 3 subfamilies, the *Alpha-, Beta-,* and *Gammaherpesvirinae*.[1] *Alphaherpesvirinae* include herpes simplex virus types 1 and 2 (HSV-1 and HSV-2, respectively) and VZV. HSV-1 and HSV-2 cause mucocutaneous ulcerative disease, keratitis, encephalitis, and severe neonatal disease.[2] VZV causes varicella (chickenpox) as the primary infection and shingles as the reactivated infection.[3] Both HSV and VZV can cause severe disseminated and at times fatal disease, rarely in individuals with apparently normal immune systems but more often in immunocompromised hosts.

Betaherpesvirinae include human cytomegalovirus (CMV), human herpesvirus 6 (HHV6), and human herpesvirus 7. CMV primary infections are frequently asymptomatic; CMV is the most common congenital infection in developed countries, however, and is the leading nongenetic cause of sensorineural hearing loss, as well as being a frequent cause of serious complications in immunocompromised individuals.[4] HHV6 causes a self-limiting febrile exanthem in young infants known as roseola. However, it is in the posttransplant patient population that both HHV6 and human herpesvirus 7 frequently reactivate and seem to be associated with significant posttransplant complications, including encephalitis and interstitial pneumonitis.[11]

The *Gammaherpesvirinae* include Epstein-Barr virus (EBV), human herpesvirus 4, and Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8. EBV causes a systemic illness known as infectious mononucleosis and is strongly associated with multiple malignancies, including nasopharyngeal carcinoma, Burkitt lymphoma, Hodgkin lymphoma, gastric carcinoma, and posttransplant lymphoproliferative disorders (PTLDs).[12] PTLDs are a complication seen in recipients of both solid and hematopoietic bone marrow transplant. Advanced forms of the disease have a high mortality despite treatment, which is primarily chemotherapy. Current strategies involve close monitoring of EBV viremia as an early signal for the development of PTLDs, with improved mortality with pre-emptive chemotherapy. Some earlier studies suggested that prophylactic use of either acyclovir or ganciclovir could reduce the incidence of EBV-associated PTLD.[13–16] However, a recent meta-analysis, which included 31 studies, found that the use of antiviral prophylaxis in high-risk EBV-naive patients had no effect on the incidence of PTLD in solid organ transplant recipients.[17] KSHV is a human tumor virus associated with Kaposi sarcoma and 2 lymphoproliferative disorders, primary effusion lymphoma and multicentric Castleman disease, both occurring primarily in individuals with AIDS.[18]

Specific Patient Populations

Zoster a significant disease burden in both immunocompetent and immunocompromised populations. Since the introduction of the varicella vaccine, the incidence of varicella has dramatically decline in the United States, with a decreased risk of developing zoster.[19] A recently approved zoster vaccine will more than likely continue to reduce the prevalence of these infections.[20] Attempts at developing a successful HSV vaccine, however, remain elusive,[21] and a considerable number of individuals experience either recurrent labial, genital, or eye disease. Episodic treatment with antiviral medications for reactivated disease, together with prolonged courses of antiviral agents for suppression, remains the cornerstone of treatment.

Neonates are a unique population to consider. CMV is the most common congenitally acquired[22] infection in developed countries. Fifteen percent of these infections will have significant sequelae apparent at birth. Of the remaining 85% with apparently no symptoms at birth, up to 15% of these will develop some degree of sensorineural hearing loss over the next 5 to 6 years. In terms of neonatal HSV infection, the incidence of disease is estimated to be between 10 and 60/100,000 live births in the United States.[23,24] Untreated neonatal HSV disease has a high morbidity and mortality rate.

Immunocompromised hosts are also at increased risk. Severe disseminated herpesvirus infections were a significant problem in patients with AIDS before successful combined antiretroviral therapies and preservation of immune function; since the advent of these treatment practices, the prevalence has substantially decreased. With the increasing number of individuals undergoing solid and hematopoietic stem cell transplantation, there is a growing need for new antiviral agents and consideration of combination therapies, as these infections result in severe disease and increased mortality, especially with the development of antiviral resistance. The incidence of CMV reactivation is reportedly between 40% and 80% in CMV-seropositive allogeneic hematopoietic stem cell transplant (alloHCT) recipients not receiving antiviral

prophylaxis. This viremia is linked to several negative outcomes, including increased mortality due to CMV disease, increased incidence of graft-versus-host disease (GVHD), an increased risk of posttransplant malignancy relapse, and mortality linked to the side effects of currently approved antiviral agents, notably myelosuppression and renal failure.[25]

Burden of Antiherpesviral Resistance

Although antiherpesviral resistance has been documented in immunocompetent individuals, the prevalence remains low. For example, in individuals receiving long-term suppressive therapy with acyclovir for recurrent genital HSV, the prevalence of resistance ranges from 0.1% to 0.7%.[5] A higher prevalence rate (6.4%) for acyclovir resistance has been documented in immunocompetent individuals receiving suppressive therapy for herpes keratitis, likely secondary to the cornea being an immune-privileged site.[26] In general, antiherpesviral resistance rates are higher in immunocompromised individuals. The prevalence of HSV infections with reduced susceptibility to acyclovir varies depending on the immunocompromising condition from between 3.5% and 7% in HIV-positive patients,[27,28] to 2.5% and 10% in transplant recipients.[27] There have been a few reports of even higher rates in individuals undergoing lymphocyte depletion regimens for alloHCT.[6,29]

In patients undergoing transplant, the prevalence of CMV resistance ranges from 1.5% to 9.5%.[7,8,30] CMV resistance has been documented in pediatric alloHCT, with one prospective study showing that 63.3% and 60.9% of subjects already had evidence of resistance-associated sequence variants in CMV genes *UL97* and *UL54*, respectively, before initiation of antiviral therapy; despite this finding, CMV-resistant infection due to a *UL97* sequence variant occurred in 4.1% of subjects and *UL54*-associated resistant infection in 2%, with an overall attributable mortality of 2%.[31] Ongoing viral replication in the setting of antiviral pressure (which occurs especially in immunocompromised hosts undergoing antiviral therapy), together with the genetic diversity of viral populations within the host, provides the optimal environment for resistance to emerge with breakthrough disease or treatment failures.[5,32]

Inadequate dosing is an important contributor to emerging antiherpesviral resistance. A retrospective review of CMV infection in 51 lung transplant recipients identified CMV infection in 21 patients; of these, 11 had suboptimal response to ganciclovir, 5 were found to have suboptimal drug levels, and genotypic analysis documented ganciclovir resistance mutations in each of those 5 patients.[33] Due to the limited number of targets available among the approved antiherpesviral therapies, as well as the inherent constraints of second-line antiviral agents (including the necessity of intravenous [IV] administration and their unfavorable toxicity profiles), antiviral resistance to first-line agents such as acyclovir and ganciclovir is becoming an increasing concern.[9]

MECHANISMS OF VIRAL REPLICATION

When considering the mechanisms of action of current and future antiviral agents, it is important to have an understanding of how herpesviruses replicate. Steps in viral DNA replication for HSV have been particularly well described and are summarized here. Initiation requires binding of proteins to the DNA replication origin site. These proteins distort and destabilize the DNA strand, while the helicase-primase complex is then recruited to unwind the duplex DNA and synthesize short RNA primers to initiate DNA replication. Once the polymerase complex is recruited to the replication fork, it catalyzes leading and lagging strand DNA synthesis. Interaction with host cell repair/recombination pathways are believed to be involved in the production of viral concatemeric DNA, which is an essential step for the generation of progeny virus. The packaging machinery recognizes longer-than-unit-length concatemers during encapsidation. HSV infection also results in dramatic reorganization of the nucleus of the infected host cell. This process involves the relocalization of cellular proteins and the ordered assembly of replication compartments, which are large globular domains within the nucleus of infected cells in which gene expression, DNA replication, and cleavage and packaging are believed to occur.[34] Although each virus is unique, analogous proteins and replication pathways have been identified across the herpesvirus family. As viral pathogenesis and mechanisms of viral replication are elucidated, novel targets for antivirals are being developed.

ANTIHERPESVIRAL TARGETS

DNA Polymerase

The mainstay agents currently licensed to treat herpesvirus infections all share a common mechanism of action by inhibiting DNA polymerase. The majority are nucleoside analogues, which act as competitive inhibitors for naturally occurring nucleosides or nucleotides that are used by the viral DNA polymerases to transcribe the viral DNA chain. Each ultimately is incorporated into the growing viral DNA chain and either result in chain termination or significant slowing and inhibition of DNA polymerase activity. These agents therefore require actively replicating virus to be able to exert their effect and have no effect on nonreplicating latent virus.[35,36] Antiviral agents targeting DNA polymerase in this manner include acyclovir, penciclovir, valacyclovir, famciclovir, valomaciclovir, ganciclovir, valganciclovir, cidofovir, and brincidofovir. Foscarnet also exerts its antiviral effect on the viral DNA polymerase, but it does so through a different mechanism of action; it is a pyrophosphate analogue that binds directly to DNA polymerase and interferes with the pyrophosphate binding required for DNA polymerase activity, thus interfering directly with DNA polymerase.[37]

Terminase Complex

Another target site that has been exploited is the CMV terminase complex, the inhibition of which interferes with viral packaging. This complex is required for the cleaving of the CMV genome units generated in tandem and then packaged into preformed virus capsids.[38] The terminase complex inhibitor letermovir has recently been approved for posttransplantation suppression of CMV in adults.[39,40]

Helicase-primase Complex

Antiviral agents with activity against HSV are being developed by exploiting a new target known as the helicase-primase complex, which is a heterotrimer comprising protein subunit products of *UL5* (helicase), *UL52* (primase), and *UL8* (ancillary protein). This complex interacts with other components of the replication machinery to coordinate replication fork progress.[41] Antiviral agents (including pritelivir and amenamevir) target this complex, inhibiting the unwinding, priming, and ultimately the replication of the DNA template.[42]

Protein Kinase

Lastly, the CMV UL97 protein kinase, which plays a role in viral encapsidation and nuclear egress of viral particles from infected cells, has been targeted with the development of maribavir.[43]

ANTIHERPESVIRAL AGENTS

With foundational understanding of viral replication and the potential targets of therapeutic intervention, this section considers antiviral agents that exhibit an inhibitory effect on some or all of the herpesviruses. Agents with approved indications are considered first (**Table I**), followed by select agents currently undergoing clinical investigation (**Table II**).

Antiherpes Viral Agents Currently in Clinical Use

Acyclovir, Valacyclovir, Penciclovir, and Famciclovir: Indicated for Treatment of HSV-1, HSV-2 and VZV

Acyclovir and the related compounds penciclovir, valacyclovir, and famciclovir are active against HSV and VZV. These agents are nucleoside analogues that are preferentially taken up by virally infected cells over uninfected cells. Once inside the cell, the molecule undergoes a first step of phosphorylation by the viral-encoded enzyme thymidine kinase. It is then converted to its active triphosphate form by cellular kinases. The active triphosphate exhibits greater selective inhibition of the viral DNA polymerase than it does of the cellular polymerase, incorporating into the growing viral chain. Acyclovir triphosphate results in chain termination and cessation of viral replication.[35,44,45] Penciclovir cannot properly be considered an obligate chain terminator owing to the presence of a 3′-hydroxyl group on its acyclic side chain, which can allow for a limited amount of continued chain elongation, although still causing significant inhibition of DNA polymerase.[5]

Acyclovir is available as a topical, oral, or IV medication. Oral acyclovir has poor bioavailability, with only 15% to 30% of the dose being absorbed. The development of valacyclovir, the L-valyl ester of acyclovir, has overcome this limitation, with >50% of the dose being absorbed in adults and achievable plasma levels that are comparable to the IV administration of acyclovir.[46,47] Acyclovir and its related compounds have a very favorable side-effect profile and are generally well tolerated even with long-term administration. The most frequent serious side effect is kidney

Table I. Antiherpesviral agents currently licensed for use.

Drug	Antiviral Activity	Mechanism of Action	Approved Clinical Indications
Acyclovir (valacyclovir)	All herpesviruses	Nucleoside analogue—polymerase inhibitor	Treatment and suppression of HSV and VZV infections
Penciclovir (famciclovir)	HSV VZV	Nucleoside analogue—polymerase inhibitor	Treatment of zoster and treatment and suppression of genital HSV Penciclovir topical for HSV labialis
Ganciclovir (valganciclovir)	All herpesviruses	Nucleoside analogue—polymerase inhibitor	Treatment and suppression of CMV infections
Foscarnet	All herpesviruses	Pyrophosphate analogue—polymerase inhibitor	Treatment of acyclovir- or ganciclovir-resistant HSV, VZV, and CMV infections
Cidofovir	All herpesviruses	Nucleotide analogue—polymerase inhibitor	Treatment of acyclovir-, ganciclovir-, and foscarnet-resistant HSV and CMV infections
Letermovir	CMV	Terminase complex inhibitor; inhibits cleavage of CMV genome units and viral particle packaging	Suppression of CMV infection posttransplantation

CMV = cytomegalovirus; HSV = herpes simplex virus; VZV = varicella zoster virus.

toxicity,[48–50] whereas neutropenia can occur with high-dose acyclovir,[51] and neurotoxicity has rarely been described.[48]

Famciclovir is the diacetyl ester prodrug of penciclovir and markedly improves bioavailability, allowing oral administration. Currently, penciclovir is only available in topical form. Penciclovir is ~100-fold less potent than acyclovir against HSV; however, due to high intracellular concentrations and a long $t_{1/2}$, it remains an effective antiviral agent.

Acyclovir has some degree of in vitro inhibitory activity against most of the human herpesviruses, with clinically achievable inhibition shown in particular against HSV-1, HSV-2, and VZV. A series of randomized placebo-controlled trials established the superiority of acyclovir over vidarabine (the first licensed systemic antiherpesviral therapy) for HSV encephalitis,[52] disseminated VZV in immunocompromised hosts,[53] and neonatal HSV infections,[51,54,55] making it the first-line antiviral agent for these infections.[56] Acyclovir also is safe to administer in the third trimester of pregnancy to reduce HSV recurrence and viral shedding at time of delivery, although it does not fully prevent neonatal HSV disease.[57,58] In the United States, the only pediatric indications for which valacyclovir is licensed for therapeutic use are orolabial HSV recurrences in children aged ≥12 years and chickenpox in children aged 2 to 17 years.[59]

Over the past 2 decades, both HSV and VZV have developed resistance to acyclovir. Viral resistance usually occurs through mutations in the thymidine kinase gene (*UL23*) and less frequently in the gene that encodes the catalytic subunit of the DNA polymerase (*UL30*).[32] Acyclovir resistance occurs most frequently in immunocompromised patients[60,61] but has been reported in immunocompetent hosts undergoing chronic suppressive acyclovir therapy for genital herpes[62] and in neonates.[63,64] Penciclovir-resistant HSV isolates have mutations that map primarily to *UL23*, although isolates with mutations in *UL30* have been identified. Not surprisingly, there is a significant amount of cross-resistance between acyclovir and

Table II. Antiherpesviral agents under development.

Drug	Antiviral Activity	Mechanism of Action	Study Population/Stage of Study
Brincidofovir	All herpesviruses	Nucleotide analogue—polymerase inhibitor	Phase III study reported increased CMV disease possibly related to increased GVHD.* CMV trials suspended; adenovirus trials ongoing. IV preparation in development
Maribavir	CMV	Competitive inhibitor of ATP binding to UL97 protein kinase inhibition of viral encapsidation and nuclear egress of viral particles	Phase III study failed to show efficacy for suppressive therapy to prevent disease post-BMT.[122] Ongoing Phase III trial to determine efficacy for treatment of resistant CMV disease in transplant recipients (NCT02931539)
Valomaciclovir	HSV VZV EBV	Nucleoside analogue—polymerase inhibitor	Completed small, randomized, placebo-controlled trial to assess clinical activity for infectious mononucleosis due to EBV. Phase IIb trial for treatment of zoster in adults underway[123]
N-Methanocarbathymidine	HSV EBV KSHV	Nucleoside analogue—polymerase inhibitor	Demonstrated superior efficacy in animal models of HSV infection.[127,128] Ongoing Phase I trial in healthy volunteers (NCT02778386)
Pritelivir (AIC316)	HSV	Helicase-primase inhibitor	In vitro potency superior to acyclovir and effective in animal models of HSV infection.[129,130] Completed Phase II trial for treatment of genital herpes.[132] Ongoing open-label trial to assess efficacy and safety for treatment of acyclovir-resistant mucocutaneous HSV infection in immunocompromised hosts (NCT03073967)
Amenamevir (ASP2151)	HSV VZV	Helicase-primase inhibitor	Approved for treatment of zoster in Japan in September 2017. In the United States, Phase I trial halted due to safety concerns; no further data available

ATP = adenosine triphosphate; BMT = bone marrow transplantation; CMV = cytomegalovirus; EBV = Epstein-Barr virus; GVHD = graft-versus-host disease; HSV = herpes simplex virus; IV = intravenous; KSHV = Kaposi's sarcoma-associated herpesvirus; VZV = varicella zoster virus.

* Source: Marty FM, Winston DJ, Chemaly RF, Boeckh M, Mullane KM, et al. 2016. Brincidofovir for prevention of cytomegalovirus (CMV) after allogeneic hematopoietic cell transplantation (HCT) in CMV-seropositive patients: a randomized, double-blind, placebo-controlled, parallel-group phase 3 trial [abstract]. *Biol Blood Marrow Transplant* 22: S23.

penciclovir resistance mutations.[65] Most acyclovir-resistant VZV strains have been isolated from HIV-infected children and adults who have profoundly depleted CD4 counts and have been undergoing chronic suppressive acyclovir therapy.[66] Foscarnet is the drug of choice for acyclovir-resistant strains of VZV and HSV.[63]

Ganciclovir and Valganciclovir: Indicated for Treatment of CMV, With Activity Against All Herpesviruses

Ganciclovir, as with acyclovir, has the first step of phosphorylation carried out by a virally encoded kinase, with the second and third phosphorylation being catalyzed by host cellular enzymes. Rather than the thymidine kinase of HSV and VZV, CMV has a phosphotransferase protein known as UL97 kinase, which is encoded by *UL97*.[67] Ganciclovir is also preferentially taken up by CMV-infected cells, and the intracellular $t^1/_2$ is >24 hours. After phosphorylation, ganciclovir triphosphate acts as a competitive inhibitor of viral DNA polymerase by being incorporated into the growing viral DNA chain, resulting in slowing and cessation of DNA chain elongation.[36] It has greatest activity against CMV. It also has activity against HSV-1 and HSV-2 that is comparable to acyclovir and is almost as active as acyclovir against VZV.[68,69] Ganciclovir is indicated for the treatment of CMV infections in immunosuppressed patients with systemic or ocular CMV disease. It is also used for the suppression of CMV retinitis and prevention of CMV disease in transplant patients.[70] In addition, its oral prodrug valganciclovir is indicated for the treatment of infants with symptomatic congenital CMV infection.[71]

Ganciclovir is generally administered via the IV route because oral absorption is poor. After administration, adequate levels are achieved in the eye, cerebrospinal fluid, and brain tissue. Most of the dose is eliminated unchanged in the urine, requiring proportional dose adjustment in renal impairment.[36] The pharmacokinetic parameters of ganciclovir are essentially the same in the neonatal population.[72] Valganciclovir is the L-valine ester prodrug of ganciclovir, which improves the bioavailability, exceeding 60% after oral administration.[73,74] Oral valganciclovir produces exposures to ganciclovir similar to those reported after IV administration of ganciclovir,[75] even in infants.[76]

Ganciclovir has some activity against cellular DNA polymerases and the potential for incorporation into host cellular DNA, which accounts for its associated toxicity. The main toxicity is myelosuppression, with dose-dependent neutropenia being the most commonly reported.[70] Owing to the myelosuppression, many post-transplant protocols, especially those for hematologic malignancies, avoid suppressive therapy with valganciclovir and rather monitor for CMV viremia and treat pre-emptively. Relevant to its use in neonatal and infant populations, ganciclovir has been shown in preclinical analyses to be mutagenic, carcinogenic, and teratogenic and has been reported to cause irreversible reproductive toxicity in animals. None of these effects has been shown in humans to date, but the long-term safety profile in infants and children treated with ganciclovir or valganciclovir has not been fully established.[69,71,72]

Ganciclovir resistance in CMV strains has been identified in both laboratory and clinical isolates. The most common mechanism of resistance is due to mutations in *UL97*.[77] Another mechanism of resistance is due to mutations in the CMV DNA polymerase gene. Mutations in the thymidine kinase gene that confer acyclovir resistance in HSV strains will also confer resistance to ganciclovir.[68] Ganciclovir-resistant isolates of HSV due to mutations in DNA polymerase have also been identified. The mechanism of resistance to ganciclovir and valganciclovir is the same.[78] As with most antimicrobial agents, low concentrations of drug exposure results in selective pressure for the development of resistance, as indicated by the higher rate of resistance seen after the use of poorly absorbed oral ganciclovir compared with IV ganciclovir.[79] With the improved bioavailability of valganciclovir, the rate of resistance is the same among oral valganciclovir and IV ganciclovir recipients.[80,81]

Foscarnet: Activity Against All Herpesviruses

Foscarnet is an inorganic pyrophosphate analogue that directly inhibits DNA polymerase by blocking the pyrophosphate binding site and preventing cleavage of pyrophosphate from deoxynucleotide triphosphates.[37] It is a noncompetitive inhibitor of viral DNA polymerase and is not incorporated into the growing viral DNA chain. It is ~100-fold more active against viral enzymes than against host cellular enzymes. Foscarnet has shown activity against all known human herpesviruses, including most acyclovir-resistant HSV and VZV strains and ganciclovir-resistant CMV isolates.[82]

Currently, foscarnet is approved for the treatment of CMV retinitis in patients with AIDS and for the

treatment of acyclovir-resistant mucocutaneous HSV infections in immunocompromised patients. It has shown efficacy for the treatment of ganciclovir-resistant CMV retinitis in patients with AIDS[83] and has been administered in combination with ganciclovir in refractory cases of chorioretinitis.[84] The safety and efficacy of foscarnet have yet to be established for the treatment of other types of herpesvirus infections.[85]

Foscarnet is only available as an IV medication. Data regarding tissue distribution are limited, but cerebrospinal fluid concentrations are about two thirds of those of serum.[86] The serum $t^1/_2$ is 48 hours, and most of the dose is eliminated unchanged in the urine. Careful monitoring of renal function is required, with dosage adjustments proportional to the degree of reduction in creatinine clearance. Foscarnet is nephrotoxic, with elevations of serum creatinine occurring in 50% of patients by the second week of therapy. Acute kidney injury can result from acute tubular necrosis or interstitial nephritis. In most affected patients, kidney function will return to normal in 2 to 4 weeks after discontinuing the medication.[87] In addition, foscarnet can result in multiple electrolyte disturbances and requires close laboratory monitoring.[88]

Foscarnet-resistant isolates of both HSV and CMV can be readily selected in vitro by passage of wild-type virus in the presence of increasing concentrations of the drug. All resistant mutants are known to be generated through mutation in the viral DNA polymerase gene. Strains of CMV, HSV, and VZV with 3- to 5-fold reduced sensitivity to foscarnet have been reported. These isolates may respond to treatment with acyclovir or cidofovir.[89,90]

Cidofovir: Activity Against All Herpesviruses

Cidofovir is an acyclic phosphonate nucleotide analogue. Unlike acyclovir and ganciclovir, it already has a single phosphate group attached, and thus a viral kinase is not required for initial phosphorylation. Cellular kinases phosphorylate the molecule to cidofovir-diphosphate, which is incorporated into the genomic DNA of the virus. Viral DNA polymerase exhibits a 25- to 50-fold greater affinity for cidofovir-diphosphate compared with host cellular polymerase, thereby leading to selective inhibition of viral DNA replication.[91,92] Cidofovir has shown in vitro activity against all human herpesviruses, polyomavirus, orthopoxviruses, adenovirus, BK virus, and human papillomavirus. Cidofovir is indicated for the treatment of immunocompromised hosts with HSV and CMV infections that have developed resistance to acyclovir, ganciclovir, and foscarnet.[93,94] In addition, cidofovir has shown efficacy in treating adenovirus[95–97] and BK virus[98,99] in immunocompromised hosts. Cidofovir can only be administered intravenously. It concentrates in renal cells 100 times more than in other tissues, resulting in damage to the proximal tubules. Renal toxicity manifests as proteinuria and glycosuria.[100–102] To decrease nephrotoxicity, aggressive IV prehydration and probenecid are used with each cidofovir dose.[103]

Only a small number of cidofovir-resistant CMV isolates have been described. Some cidofovir-resistant CMV isolates are also resistant to ganciclovir because of mutations within the DNA polymerase gene but remain sensitive to foscarnet.[104,105]

Letermovir: Activity Against CMV

Letermovir inhibits the terminal phase of the CMV life cycle by targeting the subunit *UL56* of the terminase enzyme complex.[106,107] This terminase complex, which involves proteins coded for by *UL51*, *UL56*, and *UL89*, is required for cleavage of the multimeric CMV genome units generated in tandem before being packaged into preformed virus capsids.[38] Letermovir's antiviral activity is highly specific to CMV and currently is the most active molecule against CMV, with a very low median effective concentration and preserved activity against CMV isolates that are resistant to other antiviral agents.

Letermovir was licensed in the United States in November 2017, in both oral and IV formulations, for the prophylaxis of CMV infection in adult CMV-seropositive recipients of an alloHCT. Studies that led to this approval included a Phase II trial conducted in 131 HCT recipients randomly assigned in a 3:1 ratio to 3 sequential study cohorts according to a double-blind design. Patients received oral letermovir at a dose of 60, 120, or 240 mg/d or matching placebo for 12 weeks after engraftment. The primary end point was all-cause prophylaxis failure defined as discontinuation of study drug because of CMV antigen or DNA detection, end-organ disease, or any other cause. The incidence of prophylaxis failure was inversely dose dependent, with 32% failure in the 120-mg group ($P = 0.01$) and 29% in the 240-mg group ($P = 0.007$). The study drug safety profile was similar to placebo, with no hematologic toxicity or nephrotoxicity.[39]

After this study,[39] a Phase III, randomized, double-blind, placebo-controlled trial compared letermovir with placebo in 565 CMV-seropositive alloHCT recipients aged ≥18 years.[40] Participants received letermovir administered orally or intravenously for 14 weeks after transplantation, and randomization was stratified according to trial site and CMV disease risk. Letermovir was administered at a dose of 480 mg/d (or 240 mg/d in patients taking cyclosporine). The primary end point was the development of clinically significant CMV infection through week 24 after transplantation in patients who had no detectable CMV DNA at the time of randomization. Patients were enrolled from June 2014 to March 2016. Of these, 495 patients had undetectable CMV DNA at randomization, with 325 receiving letermovir and 170 receiving placebo. In total, 122 (37.5%) developed clinically significant CMV infection in the letermovir group compared with 103 (60.6%) in the placebo group ($P < 0.001$).

The main benefits of letermovir are its good clinical and biological tolerability, its ability to be administered by an oral or IV route, and its preserved efficacy to treat resistant CMV infections. Its main limitation is its narrow spectrum of activity: it is active against CMV and has no cross-activity toward any other herpesviruses. In addition, there is a risk of rapid selection of resistant strains, as has been shown in in vitro studies.[108] The emergence of antiviral resistance in a clinical setting has only been described in 1 patient who presented with CMV viremia during the Phase II clinical study. This patient received a low dose of the study drug, and a *UL56* mutation was observed.[109]

Antiviral Agents in Development

Brincidofovir (CMX001): Activity Against All Herpesviruses

Brincidofovir is an oral, bioavailable lipid acyclic nucleotide phosphonate with the same in vitro broad-spectrum antiviral activity as cidofovir.[110,111] Brincidofovir is delivered into target cells whereupon the lipid side chain is cleaved, releasing cidofovir to be further phosphorylated by intracellular kinases to cidofovir-diphosphate, which then acts as an alternate substrate inhibitor of DNA polymerase. It is associated with >100-fold higher intracellular levels of cidofovir-diphosphate compared with cidofovir, with resultant antiviral activity up to 1000-fold against HSV, CMV, and VZV compared with cidofovir, as well as increased activity compared with ganciclovir and foscarnet.[112–116] Furthermore, synergistic inhibition of HSV replication in cell culture and in animal models has been reported when brincidofovir and acyclovir are combined.[117] In addition to enhanced antiviral activity, brincidofovir does not seem to be nephrotoxic, possibly because,[118,119] unlike cidofovir, brincidofovir is not a substrate of the human organic anion transporter 1 enzyme located in the proximal renal tubule.[111,118]

Preclinical analyses of brincidofovir were promising; however, disappointing results of a Phase III trial evaluating its use for the prevention of CMV disease in seropositive alloHCT patients have slowed its progress to market. In this trial, known as the SUPPRESS trial (clinicaltrials.gov identifier NCT01769170), 458 patients from 37 centers were enrolled and randomized to receive brincidofovir or placebo for the first 100 days' post-alloHCT. The end point was the prevention of clinically significant CMV infections 24 weeks' post-transplant. A higher CMV infection rate was observed in the brincidofovir-treated group compared with those receiving placebo (22% vs 11%; $P = 0.06$). The treatment failure observed in this study was due in part to the increased number of infections that occurred after prophylaxis was discontinued, with one factor being that an increased proportion of treatment group participants were found to have developed GVHD. During the first 14 weeks, while taking prophylaxis, significantly fewer CMV infections occurred in the brincidofovir group compared with placebo group (24% vs 38%; $P = 0.002$). Digestive symptoms were observed in a greater proportion of brincidofovir recipients, but it is unclear if this finding was related to drug toxicity or if the drug potentiated the onset of digestive GVHD. Regardless, the need for initiating immunosuppression to treat GVHD was most likely linked to the observed increase in clinically significant CMV infections in this group. Because of this study, all proposed future studies comparing oral brincidofovir for the prevention of CMV disease are currently suspended, although investigations into its utility in the treatment of other viral infections, such as adenovirus, are ongoing.

Maribavir: Activity Against CMV

Maribavir is a competitive inhibitor of ATP, binding to the UL97 protein kinase, which mediates one of the terminal steps in viral replication causing inhibition of viral encapsidation and nuclear egress of viral particles

from infected cells.[43] Maribavir is orally administered and has specific antiviral activity against CMV, including ganciclovir-resistant and cidofovir-resistant CMV strains.[120] Inhibition of the UL97 kinase by maribavir has been shown to affect the phosphorylation of ganciclovir, and thus these 2 agents are antagonistic if given concurrently.[121]

The initial clinical trials reported the tolerability of maribavir, with a lower risk of hematotoxicity compared with patients treated with ganciclovir or valganciclovir and no nephrotoxicity. Based on an earlier dose-ranging study of maribavir for the prevention of CMV infection in adult alloHCT patients, a Phase III study moved forward using the lowest of the 3 doses evaluated (100 mg BID) to investigate the efficacy of maribavir prophylaxis for the prevention of CMV disease after alloHCT. In this placebo-controlled, randomized, double-blind study, 681 adult patients undergoing alloHCT were enrolled. After engraftment, patients were stratified according to recipient CMV serostatus and conditioning regimen; they were assigned 2:1 to receive maribavir 100 mg BID or placebo for 12 weeks with weekly blood CMV surveillance. The primary end point was CMV disease within 6 months of transplantation. Incidence of CMV disease within 6 months was 4% for maribavir and 5% for placebo (odds ratio, 0.90 [95% confidence interval, 0.42−1.92]) with no significant adverse events observed. This study failed to show a difference in incidence of CMV disease, and it concluded that CMV disease as a primary end point might not be sufficient to show improvements in CMV prevention in recipients of alloHCT in the setting of pre-emptive treatment.[122]

There are, however, ongoing trials to investigate other possible uses of maribavir in transplant settings. One trial is assessing the efficacy of maribavir for the treatment of CMV infections in transplant recipients who are refractory or resistant to treatment (clinicaltrials.gov identifier NCT02931539), with an estimated completion date of May 2019. Another trial currently enrolling subjects aims to compare maribavir versus valganciclovir for the treatment of CMV infection in HCT recipients (clinicaltrials.gov identifier NCT02927067); it is projected to conclude in August 2019.

Valomaciclovir: Activity Against HSV-1, HSV-2, VZV, and EBV

Valomaciclovir is a diester prodrug of an acyclic guanosine analogue that, after cellular uptake, undergoes the initial step of phosphorylation by thymidine kinase. In vitro studies have shown that it has potent activity against replicating forms of VZV and EBV. A Phase IIb trial has been conducted for the treatment of zoster; the results indicated that valomaciclovir is well tolerated in adults and exhibits noninferiority to valacyclovir.[123] Resistance analyses in VZV showed resistance mutations in the *UL23* thymidine kinase gene, which also conferred cross-resistance to acyclovir. Valomaciclovir has also been tested for the treatment of infectious mononucleosis due to primary EBV. The results have not yet been published, but the findings were presented in 2009 at a conference and suggest reduced time of EBV viral shedding[124] (clinicaltrials.gov identifier NCT00575185).

N-Methanocarbathymidine: Activity Against HSV-1, HSV-2, EBV, and KSHV

N-Methanocarbathymidine (N-MCT) is a thymidine analogue so named because it has a pseudo-sugar moiety fixed in the Northern confirmation. After cellular uptake, it is twice catalyzed by viral thymidine kinase to its mono- and diphosphate metabolites, thus requiring host cellular kinase only for the final step of phosphorylation to its active triphosphate form. Subsequently, as with all other triphosphate nucleoside analogues, it competitively inhibits viral DNA polymerase.[125] In addition to having some antiviral activity against the orthopoxviruses, N-MCT has been shown to inhibit in vitro replication of HSV-1, HSV-2, EBV, and KSHV[126] and has also shown superior efficacy in animal models of HSV infection compared with acyclovir.[127,128] A trial is currently being conducted to evaluate the safety and plasma levels of N-MCT in healthy adults (clinicaltrials.gov identifier NCT02778386). If its safety profile is favorable, N-MCT has promising potential as an antiviral agent.

Pritelivir (AIC316): Activity Against HSV

Pritelivir is one of two compounds that are currently under development which target the HSV helicase-primase complex. Original publications referred to this agent as BAY 57-1293. Its in vitro potency was superior to acyclovir in animal models of HSV disease,[129,130] and, because it has a different mechanism of action, it remains active against acyclovir-resistant HSV isolates.[131] In addition, pharmacokinetic data from clinical studies revealed a serum $t_{1/2}$ that could allow for once-daily dosing.[129] A Phase II trial of

pritelivir in healthy adults with genital HSV-2 infection showed reduced days of viral shedding and time to resolution of genital lesions with a favorable safety profile.[132] An ongoing open-label trial is being conducted to assess the efficacy and safety of treatment of acyclovir-resistant mucocutaneous HSV infections in immunocompromised patients compared with foscarnet (clinicaltrials.gov identifier NCT03073967).

Genomic surveillance of clinical HSV isolates has shown evidence of naturally occurring low-abundance sequence variants that confer resistance to pritelivir.[133] As such, it is unsurprising that resistant HSV strains can easily be selected in vitro.[131] The majority of these resistance mutations have been identified in *UL5*; however, they have also occurred in *UL52*.[134] As clinical investigation of pritelivir moves forward, further surveillance of the emergence of antiviral resistance is necessary.

Amenamevir (ASP2151): Activity Against HSV and VZV

Amenamevir, the second of the helicase-primase inhibitors in development, is an oxadiazole phenyl derivative that has potent activity against both HSV and VZV.[135] Unfortunately, a Phase I clinical trial comparing amenamevir versus valacyclovir in healthy adults was halted due to unpublished treatment-emergent adverse events (clinicaltrials.gov identifier NCT00870441). However, due to a successfully completed trial in Japan showing efficacy of amenamevir for the treatment of zoster, it was approved for this indication in Japan in September 2017. At the time of writing, there are no active trials evaluating amenamevir in the United States.

NEW DIRECTIONS FOR NOVEL THERAPEUTICS

A novel approach that is still in the earliest stages of development is the targeting of viral genetic elements important for viral fitness with CRISPR/Cas9 genome editing techniques. Because this technique does not require active replication to function, it could potentially be used to combat both productive and latent herpesvirus infections, completely abolishing viral production from infected cells. Investigators have targeted sites in the genomes of 3 different herpesviruses (HSV-1, CMV, and EBV) using CRISPR/Cas9-mediated genome editing, and they showed complete inhibition of viral replication and, in some cases, even eradication of the viral genome from infected cells.[136,137] These applications may significantly change the infectious complications of transplant patients in the future.

CONCLUSIONS

It is currently an exciting era for development of antiviral agents as novel targets are being elucidated and new agents are under development. Many of these newer agents have the potential for improved potency and more favorable safety profiles, both of which are greatly needed in the clinical setting. As new agents make inhibition of multiple targets in the viral replication cycle possible, the potential now exists to evaluate combination therapeutic strategies similar to those that have proven successful in the management of HIV. Combination antiviral therapy for herpesvirus infections in immunosuppressed hosts is particularly appealing because it may help mitigate the need for extended therapeutic regimens and the attendant risk of developing antiviral resistance while undergoing prolonged monotherapy. In addition, these new treatments need to be tested across various patient populations and conditions, including children and neonates in whom limited treatment options currently exist.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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