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SUPPLEMENT ARTICLE

Advances in the Development of Therapeutics for Cytomegalovirus Infections

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The development of therapeutics for cytomegalovirus (CMV) infections, while progressing, has not matched the pace of new treatments of human immunodeficiency virus (HIV) infections; nevertheless, recent developments in the treatment of CMV infections have resulted in improved human health and perhaps will encourage the development of new therapeutic approaches. First, the deployment of ganciclovir and valganciclovir for both the prevention and treatment of CMV infections and disease in transplant recipients has been further improved with the licensure of the efficacious and less toxic letermovir. Regardless, late-onset CMV disease, specifically pneumonia, remains problematic. Second, the treatment of congenital CMV infections with valganciclovir has beneficially improved both hearing and neurologic outcomes, both fundamental advances for these children. In these pediatric studies, viral load was decreased but not eliminated. Thus, an important lesson learned from studies in both populations is the need for new antiviral agents and the necessity for combination therapies as has been shown to be beneficial in the treatment of HIV infections, among others. The development of monoclonal antibodies, sirtuins, and cyclopropovir may provide new treatment options.

Keywords. congenital cytomegalovirus; antiviral therapy; ganciclovir; valganciclovir; sirtuins; letermovir; cyclopropovir; maribavir; monoclonal antibodies.

Advances in the prevention and treatment of cytomegalovirus (CMV) infections are limited but well documented. Realized successes indicate a need for improved therapies, if not combinations. Only 5 CMV therapeutics are approved by the US Food and Drug Administration (FDA): foscarnet (1991), ganciclovir (1994) and its prodrug valganciclovir (2001), cidofovir (1996), and letermovir (2017). Risk/benefit considerations of foscarnet and cidofovir preclude use in neonatal and infant populations; letermovir, while licensed for adult stem cell transplant recipients, is being evaluated in children undergoing transplantation. This document provides a glimpse of the limited achievements and offers hope for further improvements.

ADVANCES IN THE TREATMENT OF CONGENITAL CYTOMEGALOVIRUS INFECTIONS

CMV infection is the leading nongenetic cause of sensorineural hearing loss (SNHL) [1–4] and the most frequent known viral cause of mental retardation [5], affecting 0.5%–0.7% of live births in industrialized countries [6–8], including 19 000 to 26 600 congenital infections annually in the United States. Approximately 2300 (10%) have symptomatic disease at delivery, of whom 35% have SNHL, up to 66% have neurologic deficits, and 4% die in the newborn period [7–11]. SNHL occurs at a lower rate among the 90% of congenitally infected neonates who are asymptomatic at delivery, but accounts for the majority of cases of hearing loss overall [7, 12]. African American neonates disproportionately suffer from congenital CMV infection at triple the rate of white neonates and 9-fold higher than Asian American neonates [13]. As estimated in Table 1, each year 4000–5000 of these babies will develop CMV-associated disabilities. Congenital CMV accounts for 21% of hearing loss at birth and 24% of all cases of hearing loss by 4 years of age [1, 14]. The overall economic burden of congenital CMV infection exceeds $3 billion annually, adjusted for 2015 dollars [15–17].

Since the 1980s, the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (CASG) has conducted a series of clinical trials with parenteral ganciclovir and oral valganciclovir in infants <1 month of age born with symptomatic congenital CMV disease [19–25].

Study Design and Results of the Ganciclovir Study

After establishing the appropriate dose of intravenous (IV) ganciclovir for use in neonates in the 1980s [19, 24, 25], the CASG conducted a phase 3 randomized controlled trial of 6 weeks of IV ganciclovir (6 mg/kg/dose IV) vs no therapy for neonates with symptomatic neonatal disease [23]. One hundred patients with symptomatic congenital CMV disease involving

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Table 1. Sequelae Following Congenital Cytomegalovirus Infection in the United States

Parameter	Estimated Annual Value
No. of live births per year	3 800 000
Rate of congenital CMV infection	0.6%
No. of infected infants	22 800
No. of infants symptomatic at birth (12.8%)	2918
Symptomatic at birth, who have or develop disability (50%)	1459
No. of infants asymptomatic at birth (87.2%)	19 882
Asymptomatic at birth, who have or develop disability (13.5%)	2684
Total with congenital CMV-related disabilities	4143

Adapted from Cannon et al [18].

Abbreviation: CMV, cytomegalovirus.

the central nervous system (CNS) were randomized. The primary study endpoint was improved brain stem evoked response (BSER) audiometry by 1 gradation between baseline and the 6-month follow-up (or, for those patients with normal hearing at baseline, normal BSER at both time points). Clinical and laboratory improvement constituted secondary endpoints. Audiological analyses were performed on the best evaluable ear ("functional" assessment) and on total evaluable ears ("biologic" assessment).

For infants available at follow-up, 21 of 25 (84%) ganciclovir recipients either had improvement in hearing in their best ear between baseline and 6 months or had normal hearing at both time points, compared with 10 of 17 (59%) subjects in the no treatment group (odds ratio [OR], 5.03 [95% confidence interval {CI}, .84–45.94]; adjusted P = .06). None of the 25 ganciclovir recipients had hearing deterioration in their best ear between baseline and 6 months, compared with 7 of 17 (41%) subjects in the no treatment group (OR, 21.11 [95% CI, 2.84–∞]; adjusted P < .001). Five of 24 (21%) ganciclovir recipients had worsening in hearing in their best ear between baseline and ≥1 year, compared with 13 of 19 (68%) subjects in the no treatment group (OR, 10.26 [95% CI, 1.79–81.92]; adjusted P = .002). Ganciclovir-treated subjects also normalized alanine aminotransferase (ALT) more rapidly compared with the no treatment group (19 vs 66 days, respectively; P = .03).

Twenty-nine of 46 (63%) ganciclovir-treated subjects developed grade 3 or 4 neutropenia (Division of AIDS Toxicity Tables) compared with 9 of 43 (21%) subjects in the no treatment group (P < .01). Fourteen of the 29 (48%) required dosage adjustments, although only 4 patients discontinued drug permanently. Neutropenia in ganciclovir-treated subjects resolved in 12.8 (±13.6) days, and in the no treatment group in 14.2 (±13.5) days. All subjects resolved their neutropenia. The incidence of grade 3–4 thrombocytopenia was comparable across both study arms, as was the incidence of grade 3–4 increases in ALT, and total bilirubin levels.

Developmental Analyses

Denver Developmental Evaluations were performed at 6 weeks, 6 months, and 12 months [26]. At the 6-week assessment, the average number of developmental delays per subject was 1.5 for ganciclovir and 2.05 for the no treatment subjects (P = .13); at 6 months, delays were 4.46 and 7.51, respectively (P = .06) and at 12 months were 9.78 vs 17.14, respectively (P = .007). Multivariate analysis of variance tested independent factors related to poor development, indicating that the ganciclovir benefit remained statistically significant at 12 months (P = .007), and approached statistical significance at 6 weeks (P = .08) and 6 months (P = .08).

Study Design and Results of the Valganciclovir Therapy Study

Ganciclovir results suggested that therapeutic benefit waned over the first 2 years of life [23], implying that a new strategy of extending the duration of therapy may be beneficial. Thus, the CASG developed a pharmacokinetic and pharmacodynamic study to determine the dose of oral valganciclovir that achieved systemic ganciclovir exposure equivalent to IV ganciclovir [21], generally an area under the curve (AUC_{12}) in the range of 27–32 μg × hour/mL, with a coefficient of variation in the range of 30%–40%.

Twenty-four subjects <1 month of age with symptomatic congenital CMV disease were assessed. A dose of 15.62 mg/kg provided an AUC_{12} of 27.4 μg × hour/mL. The oral bioavailability of valganciclovir increased in early infancy from 48% at approximately 4 weeks of life to 64% at approximately at 7 weeks of life, an increase proportionate to improved ganciclovir renal clearance.

Using the oral dose of valganciclovir of 16 mg/kg/dose twice daily, the CASG study assessed whether longer-term therapy provided prolonged beneficial effects. Ninety-six subjects were randomized to blinded study medication (47 drug, 49 placebo) after receiving 6 weeks of valganciclovir. Total ears from subjects receiving 6 months of valganciclovir were more likely to have improved hearing or to maintain normal hearing between baseline and 12 months (compared to 6 weeks) after a priori adjustment for CNS involvement at baseline (adjusted OR [aOR], 3.04 [95% CI, 1.26–7.35]; P = .01). Similar results were evident when prematurity and age at treatment initiation were added to the model (P = .01). The relative risk for improved or protected total ear hearing between baseline and 12 months for the 53 subjects with baseline CNS involvement who received 6 months of treatment and 6 weeks of treatment was 1.66 (95% CI, .92–2.4), and the risk difference was 0.27 (95% CI, .09–.45). The benefit of longer-term therapy in the total ear analysis was maintained at 24 months, with improved outcomes after adjusting for CNS involvement at baseline (aOR, 2.61 [95% CI, 1.05–6.43]; P = .04). Similar results were evident when prematurity at treatment initiation was modeled (P = .04). The relative risk for improved or protected total ear hearing between baseline and 24 months was 1.46 (95% CI, .87–2.05); the risk difference was 0.23 (95% CI, .05–.41), following 6 months of therapy.

Adjusting a priori for CNS involvement, valganciclovir therapy led to higher Bayley III Language Composite ($P = .0046$) and Receptive Communication Scale ($P = .0031$) developmental scores at 24 months compared with subjects randomized to 6 weeks of treatment. These differences were maintained when age at treatment initiation and prematurity were added to the model ($P = .0037$ and $P = .0027$, respectively).

Collectively, these trials led the FDA to modify the valganciclovir package insert to cite the dose used in CASG studies [22, 27], and the American Academy of Pediatrics now recommends 6 months of oral valganciclovir therapy for infants with symptomatic congenital CMV disease [28, 29].

Implications for Potential Biomarkers on New Treatment Approaches

Overall clinical benefit is modest, raising the possibility that more effective drugs or treatment regimens could provide additional benefit. Subjects with symptomatic congenital CMV disease who achieve complete viral suppression (defined as ≤2.5 log) by day 14 of valganciclovir therapy and maintain it over the next 4 months are statistically more likely to have improved hearing across the first 2 years of life (Table 2) [30]. However, the ability of ganciclovir/valganciclovir to cause such a rapid decline is limited to a small number of patients; typically, viral load decreases by only 1 log over the first week and then <0.5 log over subsequent weeks (Figure 1) [22]. The rapidity of clearance of virus and cerebrospinal fluid findings require further evaluation. Combination therapy has the potential to significantly advance management options in the treatment of symptomatic congenital CMV disease, as has been the case with combination therapy in the treatment of human immunodeficiency virus (HIV) and hepatitis C virus infections. These data foreshadow the potential for linking CMV biomarker(s) to drug exposure. These relationships would serve to isolate the most important pharmacokinetic parameters linked to treatment response, and more precisely identify the optimum dosing strategy.

Current CASG Studies Assessing Additional Populations and Treatment Approaches

The CASG is currently evaluating oral valganciclovir therapy when started beyond the first month of life for treatment of infants and toddlers with CMV-associated hearing loss in a placebo-controlled trial (ClinicalTrials.gov identifier NCT01649869). Furthermore, screening of 50 000 babies has been undertaken to identify approximately 230 otherwise asymptomatic infected infants who will receive oral valganciclovir therapy for 4 months (ClinicalTrials.gov identifier NCT03301415) to determine if hearing loss can be prevented.

UTILIZATION OF LETERMOVIR IN THE PREVENTION OF CMV INFECTIONS IN STEM CELL TRANSPLANT RECIPIENTS

CMV is the most common clinically significant viral infection following stem cell or organ transplantation, causing morbidity due to direct effects (pneumonia, hepatitis, retinitis and encephalitis) and indirect effects (increased risk of opportunistic bacterial and invasive fungal infections, graft-vs-host disease, delayed engraftment, or graft failure/rejection), as well as increased overall mortality [32, 33].

Two approaches exist to preventing CMV disease in transplant recipients: (1) prophylaxis, whereby antiviral treatment is started prior to viremia; or (2) preemptive therapy, defined as active surveillance for viral replication, with treatment only initiated when CMV viremia is detected. Preemptive therapy has been the preferred approach for preventing CMV disease in hematopoietic stem cell transplant (HSCT) recipients to minimize the toxicities of the available nucleoside analog agents. Although the introduction of preemptive therapy for the management of CMV infection has reduced the incidence of CMV end-organ disease, this approach has remained suboptimal as (1) preemptive therapy is initiated only after patients develop CMV viremia, and any level of viremia is associated with an increased risk of overall mortality; and (2) nucleoside analog inhibitors have toxicities, including myelosuppression, delayed bone marrow engraftment, and nephrotoxicity. Prophylaxis would be a preferred strategy if a drug had reduced toxicity and/or improved potency compared with existing agents. PREVYMIS (letermovir) was developed to meet the unmet medical need for a prophylactic anti-CMV agent with potent activity and a favorable therapeutic index that could improve clinical outcomes in HSCT recipients [34].

PREVYMIS (letermovir) is an inhibitor of the CMV viral terminase that is responsible for the cleavage of newly synthesized CMV DNA into individual unit–length viral genomes. The first terminase inhibitors described for CMV were the halogenated benzimidazole analogs and were shown to eliminate the formation of monomer viral genomes in infected cells [35, 36]. This series is remarkably specific for CMV, as reviewed earlier [37]. While they are nucleoside analogs, they do not require phosphorylation for antiviral activity [38], but rather target components of the viral terminase, including UL56 and UL89 [39]. Letermovir demonstrated potent and selective inhibition of CMV activity in vitro and in preclinical models [40–45].

A series of phase 2 and 3 studies has defined antiviral activity and safety. First, proof of antiviral activity in humans was established in solid organ transplant recipients who had CMV viremia. Second, a phase 2b study demonstrated a dose response and excellent tolerability in the prevention of CMV viremia and/or disease in HSCT recipients. Last, these findings were confirmed in a pivotal randomized, placebo-controlled phase 3 study for the prophylaxis of CMV infection/disease in adult CMV-seropositive recipients of an allogeneic HSCT in the first 3 months posttransplant—a time frame for highest risk of CMV reactivation. Letermovir prophylaxis, started within 28 days and continued until 14 weeks posttransplant, effectively

Table 2. Improvement and Protection in Best-Ear and Total-Ear Hearing Between Baseline and Follow-up in Subjects With Complete Viral Suppression by Day 14 of Therapy Through Month 4, and Subjects Without Complete Viral Suppression

Analysis	No. of Subjects or Ears	Hearing Between Baseline and Follow-up		*P* Value
		Improved/Protected	Others	
6-mo total-ear hearing				
With complete viral suppression	19	17 (89)	2 (11)	.0098
Without complete viral suppression	48	27 (56)	21 (44)	
12-mo total-ear hearing				
With complete viral suppression	20	20 (100)	0	.0007
Without complete viral suppression	48	30 (63)	18 (38)	
24-mo total-ear hearing				
With complete viral suppression	17	16 (94)	1 (6)	.0458
Without complete viral suppression	41	28 (68)	13 (32)	

Adapted from Marsico et al [30].

prevented clinically significant CMV infection/disease, with 37.5% of participants on letermovir developing CMV viremia/disease compared to 60.6% participants on placebo at 24 weeks posttransplant ($P < .0001$). These results demonstrated an approximate 40% relative reduction of CMV infection/disease compared to placebo with a number needed to treat to prevent 1 case of infection/disease of 5 patients. Furthermore, all-cause mortality was decreased by approximately 30% compared to placebo (10.2% letermovir group vs 15.9% placebo group) at week 24 post-HSCT, with a number needed to treat of 20. The mortality benefit of letermovir treatment was maintained through week 48 after transplantation. Letermovir was well tolerated with no evidence of myelotoxicity; participants who received letermovir had a similar time to engraftment compared with those who received placebo [46].

PREVYMIS was approved in the United States by the FDA on 9 November 2017 and by the European Union on 8 January 2018, after accelerated assessment. As the first CMV therapeutic approved since 2001, PREVYMIS marks the beginning of a new era for the prophylaxis of CMV infection and disease in HSCT recipients by enabling the adoption of a new prevention paradigm of CMV management.

DRUGS IN CLINICAL DEVELOPMENT

Maribavir, a benzimidazole L riboside structurally related to the halogenated benzimidazole terminase inhibitors, is a highly specific inhibitor of the CMV UL97 kinase, as reviewed [47, 48]. This molecule exhibits favorable pharmacokinetic properties, is well tolerated, and holds promise for the treatment of CMV infections [49–51]. In a phase 3 study, maribavir-treated patients failed to meet the clinical endpoints [52], possibly due to an inadequate dose. One phase 3 trial recently demonstrated equivalence of maribavir at higher doses compared with ganciclovir; another is ongoing (ClinicalTrials.gov identifiers NCT02931539 and NCT02927067) [53]. The inhibition of UL97 kinase activity by this drug would interfere with the activation of ganciclovir and filociclovir; thus, their concomitant administration would likely reduce the efficacy of the later dugs

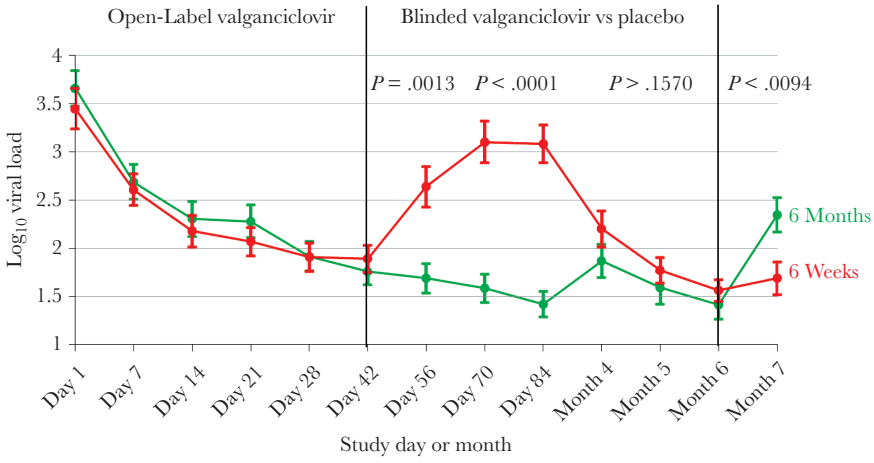

Figure 1. Whole blood cytomegalovirus DNA viral load. Reprinted from Kimberlin et al [31] with permission. Clearance of virus in congenitally infected infants who received either 6 weeks or 6 months of therapy. A rebound in virus titer occurred upon drug discontinuation.

[54–56]. Clinical studies need to be designed with this issue in mind, but strategies to minimize this effect clearly exist.

The acyclic nucleoside phosphonates, such as cidofovir, have excellent antiviral activity against CMV, but its use is limited by toxicity [57, 58]. Brincidofovir, the new ether lipid ester prodrug of cidofovir [59], exhibits enhanced in vitro efficacy against all the human herpesviruses, including CMV [60], and retained activity against ganciclovir-resistant isolates of CMV having mutations in the UL97 gene. Brincidofovir also exhibits markedly enhanced activity in vitro against a spectrum of DNA viruses, including the herpesviruses, orthopoxviruses, adenoviruses, and polyomaviruses [60–63]. The drug was originally designed to improve the oral bioavailability of cidofovir and indeed proved to be readily absorbed by the oral route in pharmacokinetic studies [64]. More importantly, this modification essentially eliminated its uptake in the kidney by the organic anion transporter, thus reducing its nephrotoxicity and offering a potentially significant advantage over cidofovir [65, 66].

In clinical studies, brincidofovir performed well in a phase 2 trial for CMV infections in stem cell transplant recipients and reduced the number of CMV events, the primary endpoint, at an oral dose of 100 mg twice weekly [67]. In a phase 3, double-blind, placebo-controlled, randomized trial, it did not meet its primary endpoint (above) (ClinicalTrials.gov identifier NCT00411645) [68]. An IV formulation of brincidofovir is in phase 2 development and was designed to reduce the incidence and severity of diarrhea and increase levels of the drug in other important compartments.

THERAPIES IN EARLY DEVELOPMENT

Overview of Small Molecules

Promising molecules in early stages of development have been reported. Distinct acyclic nucleoside phosphonate analogs, such as diaminopyrimidine derivatives, have broad-spectrum antiviral activity and may exhibit reduced toxicity [69]. Nonnucleoside inhibitors of the DNA polymerase also represent an important class of potent compounds, providing promise as broad-spectrum inhibitors of the herpesviruses without liabilities associated with nucleoside inhibitors [70]. The 4-oxo-dihydroquinoline derivatives provide an example of highly potent compounds with activity against all the human herpesviruses [71, 72] and have the advantage of remaining active against ganciclovir-resistant isolates of CMV [73].

The first terminase inhibitors described for CMV were the halogenated benzimidazole analogs and were shown to eliminate the formation of monomer viral genomes in infected cells [35, 36]. This series is remarkably specific for CMV, as reviewed previously [37]. Although nucleoside analogs, they do not require phosphorylation for antiviral activity [38], but rather target components of the viral terminase including UL56 and UL89 [39]. An advanced candidate is GW275175X, which inhibits the terminase complex [74] and retains good activity against CMV [75, 76].

Filociclovir, another acyclic nucleoside, is in clinical development for the treatment and/or prevention of CMV, human herpesvirus 6, and adenovirus. Filociclovir demonstrated good in vitro and in vivo potency against human CMV. Filociclovir has a dual mechanism of action, inhibiting both the CMV UL54 DNA polymerase and the CMV UL97 kinase while retaining activity against ganciclovir-resistant CMV, despite sharing a similar mechanism of action [55]. In addition, filociclovir is converted more efficiently in infected cells to the active triphosphate form than ganciclovir [77].

Oral bioavailability of filociclovir following single oral doses in rats and dogs was 22%–46% and 70%–91%, respectively. In safety pharmacology studies, filociclovir did not cause significant changes in respiratory, cardiovascular, or CNS parameters at doses up to 100 mg/kg. Filociclovir was not considered genotoxic. Toxicology studies established target organs similar to other drugs in the class: kidney, bone marrow, gastrointestinal tract, and testes [78].

The first-in-human, phase 1a clinical study (ClinicalTrials.gov identifier NCT01433835) [78] in healthy volunteers evaluated the safety and pharmacokinetics of filociclovir in single ascending oral doses. Forty-eight healthy volunteers (3 male, 45 female) were enrolled and completed the study. No serious adverse events were reported. The most common adverse events were dry mouth and headache. Maximum plasma concentrations (C_{max}) and area under the curve values increased following filociclovir doses of 35–1000 mg and decreased at 1350 mg; exposure was less than dose proportional. C_{max} values were 281.5–1175 ng/mL and area under the curve at last assessment (AUC_{last}) values were 946.2–5696 ng × hour/mL following administration of 35, 100, 350, 700, 1000, and 1350 mg oral doses.

A phase 1b clinical study (ClinicalTrials.gov identifier NCT02454699; Rouphael et al, unpublished data) has been completed in healthy volunteers and evaluated the safety and pharmacokinetics following administration of 7 oral daily doses (100, 350, or 750 mg) of filociclovir, and the results are being analyzed.

Safety data support advancement of filociclovir following work to improve formulation. Phase 2 clinical trials will be undertaken [69–72, 77–81].

Application of Monoclonal Antibodies for Therapeutic Purposes

Administering antibodies as an alternative to vaccines has been used in pregnancy to treat blood group incompatibilities to and prevent rubella, hepatitis, varicella, respiratory syncytial virus, and measles [82]. Advantages include more reliable and rapid achievement of efficacious plasma concentrations. Passive human immunoglobulin (HIG) immunization, which had suggested value for the prevention of congenital CMV infection (2005–2012), became controversial when a 2014 study failed to

prevent fetal infection [83]. Subsequent study of HIG pharmacokinetics established unexpectedly rapid clearance (11 days). Based on this observation, a phase 2 trial was conducted [84] in which 40 pregnant women with a primary CMV infection (9.6 weeks median gestational age) were treated with HIG biweekly rather than monthly as in previous trials. Transmission before amniocentesis at 20 weeks occurred in only 1 subject (2.5%), with 2 additional subjects infected after treatment. Including all 3 cases, the transmission rate was 7.5%, with no infected neonate symptomatic at birth. The results are significant ($P < .0001$) when compared to untreated historical controls matched for first trimester seroconversion with amniocentesis at 20 weeks (108 pregnancies), for whom the transmission rate was 35.2% and incidence of sequelae was 14%.

HIG is also effective in solid organ transplant. A meta-analysis of 11 randomized trials (N = 698; median follow-up of 12 months) concluded that HIG prophylaxis was associated with reduced CMV disease and improved survival [85]. For pediatric HSTC, CMV infection at 1 year was 13.4% for HIG-treated children vs 44.4% without HIG ($P = .001$) [86].

Replicating the activity of HIG with a monoclonal antibody (mAb) offers the prospect of improved consistency in manufacturing, lower infusion volume, improved pharmacokinetics, and reduced risk of off-target reactivity leading to toxicity. Moreover, HIG batches are not rigorously evaluated for titer to particular antigens, which may contribute to variability in efficacy. Antibodies to the CMV pentameric complex are abundant in HIG [87], although immunodominance is not necessarily correlated with neutralizing activity. The major clinical effort targeting the pentameric complex has been a mixture of 2 mAbs (RG7667), which showed promise in a phase 2 trial in renal transplant recipients by delaying onset of CMV excretion [88] but is no longer being developed, despite modest neutralizing activity.

A drawback to targeting the pentameric complex is that antibodies against it fail to neutralize infection of fibroblasts [89], whose ubiquitous distribution may be important for disease progression [90]. Antibodies to the gB fusion protein, which is essential for entry in all cell types, are also part of the natural immune response, and the adjuvanted gB/MF59 vaccine showed efficacy in a substantial number of subjects, both in transplant and congenital indications [91]. The first clinically tested mAb against gB (Theraclone: TCN-202) was well tolerated in a phase 1 trial, but development was discontinued. CSJ148 (Novartis) is a mixture of 2 mAbs (targets: gB [AD-4], an immunodominant region, and the pentameric complex) [92]. In a phase 1 trial, CSJ148 was well tolerated, but it too is no longer being advanced. Both AD-4 and the similarly immunodominant AD-1 are more variable than the AD-2 site I (which is also the least immunogenic site). TRL345 is a native human mAb that has 10-fold higher affinity than TCN-202 for this highly conserved epitope, and 6.5-fold higher potency in vitro than the anti-gB component of CSJ148 [93]. High affinity is important for the mAbs to prevent transmission by remaining bound to the virus following transcytosis across the placenta via the neonatal Fc receptor [94]. Furthermore, high affinity can prevent displacement of neutralizing antibodies to AD-2 site I by nonneutralizing antibodies to the adjacent site II present in approximately 25% of anti-CMV human serum samples [95]. Elevated titers to the TRL345 epitope is associated with reduced risk of congenital transmission [96].

TRL345 showed potent antiviral activity against 15 primary clinical isolates of diverse genotypes [97]. For several clinical strains, potency in a wide range of cell types was 10- to 50-fold better than HIG. The absence of a well-validated animal model has put a premium on studies using human placental tissue as a model. CMV replicates in trophoblast progenitor cells and impairs their capacity to self-renew and differentiate. TRL345 was approximately 10-fold more potent than HIG in these cells [98]. In an established ex vivo model in which explants of first-trimester human placenta are grown on Matrigel, TRL345 was highly effective at reducing infection [97].

TRL345 was also compared to a high-affinity antipentameric complex antibody cloned from published sequence data (mAb 1F11 binding to UL128-131A). The 1F11 mAb had more potent neutralizing activity on endothelial cells than TRL345, but it provided no protection against infection of smooth muscle cells, placental fibroblasts, or trophoblast progenitor cells [97]. Formation of multinucleated syncytia is a characteristic phenotype of CMV in vivo. In a study of syncytial spread in vitro, TRL345 was the most effective antibody in a comprehensive panel of 28 antibodies, both polyclonal and monoclonal, targeting all major CMV virion glycoproteins [99]. An additional observation from this study was that antibodies targeting epitopes in gH or gH/gL exhibited strain and cell type dependence. By contrast, the frequency of mutations in the TRL345 epitope is approximately 10-fold lower than for other envelope glycoprotein sites [100], despite continuous selective pressure (antibodies to the epitope represent approximately 1% of the anti-CMV activity in serum), suggesting that escape will be rare. TRL345 has been expressed in stably transformed Chinese hamster ovary (CHO) cells at 1.8 g/L. The no-observed-adverse-effect level in a 28-day toxicology study in Sprague-Dawley rats was 150 mg/kg/dose. At 15 mg/kg, the projected half-life in humans is 21 days. An investigational new drug application is expected to be filed in 2020.

ROLE OF SIRTUINS IN THE TREATMENT OF CMV INFECTIONS

Direct-Acting Antivirals Are Limited by Their Spectrum of Antiviral Effectiveness

Because direct-acting antivirals exert their therapeutic effect through direct interactions with a viral protein, they are also limited by drug resistance secondary to acquisition of

viral mutations disrupting the molecular interaction of the drug with the targeted viral protein. Drug-resistant clinical isolates of CMV have been identified for all marketed CMV antivirals: ganciclovir/valganciclovir, foscarnet, cidofovir, and letermovir [101]. A single mutation at UL56 C325 confers absolute resistance to letermovir with minimal impact on viral fitness [102, 103].

Host-targeted antivirals (HTAs) are directed against the host-cell processes upon which viruses are dependent. Compared to DAAs, HTAs have the potential to reduce or eliminate viral resistance, demonstrate broad-spectrum effectiveness, and provide therapeutic utility in areas of unmet medical need. First-generation HTAs, such as interferons, broadly activate the host's innate and adaptive immune responses (eg, hepatitis B and C) [104]. The clinical use of interferons is limited by toxicity. Next-generation HTAs are small molecules that target host sirtuin proteins. By modulating sirtuin-enzyme activity, these leads are predicted to reduce viral replication by restoring host-cell metabolism and intrinsic immunity within the infected cell.

The 7 human sirtuins (SIRT1–7) are nicotinamide adenine dinucleotide (NAD^+)–dependent deacylases that regulate cellular metabolism and gene activity by posttranslationally removing acyl groups from target proteins [105]. In addition to the deacetylase activity of all 7 SIRTs, SIRT4, SIRT5, and SIRT6 can also remove lipoyl, methylglutaryl, hydroxymethylglutaryl, and 3-methylglutaconyl moieties [106, 107]; malonyl and succinyl moieties; and myristoyl moieties, respectively. The extent to which proteins in the cell are acylated by the distinct fatty-acyl chains is dependent on acyl–coenzyme A metabolism, which can be selectively regulated [108]. SIRTs localize to the nucleus (SIRT1, SIRT2, SIRT6), nucleolus (SIRT7), cytoplasm (SIRT2), and mitochondria (SIRT3, SIRT4, SIRT5). The NAD^+ requirement for the deacylation reaction ties the activity of sirtuins to the metabolic capacity of the cell. As such, the emerging role of sirtuins is as sensors linking the metabolite profile of the cell to cellular signal transduction, as reviewed elsewhere [109]. Sirtuin-mediated deacylation of target proteins impacts numerous cell functions, including metabolism, cell cycle, apoptosis, stress response, DNA repair, and gene expression—all functions known to affect virus growth.

Sirtuins control cellular processes that impact the growth of many different viruses and function as elements of intrinsic immunity [110]. Modulation of sirtuins can restrict the growth of both RNA and DNA viruses. Viruses depend on host cell metabolism for energy, metabolic precursors for viral components, and cellular organization for replication, maturation, and dissemination. Not surprisingly, diverse, intracellular pathogens have been shown to directly interact with sirtuins in order to co-opt host-cell metabolism or epigenetic mechanisms into supporting the pathogen. These include *Salmonella typhimurium* [111], *Listeria monocytogenes* [112, 113], *Leishmania infantum* [114], Kaposi sarcoma–associated herpesvirus [115], HIV [116], and hepatitis B virus (HBV) [117, 118]. In particular, recent publications demonstrate the potential utility of SIRT2 inhibitors as antibacterial (*Listeria*) and antiviral (HBV) agents [112–117].

A small-molecule screen was carried out on a library of approximately 13 000 compounds to identify modulators of SIRT1, SIRT2, SIRT3, and/or SIRT6 deacetylase activity [119]. Belying the importance of metabolism on productive viral replication and the role of sirtuins as mediators of intrinsic immunity, among the 85 hits validated in the primary assay as significantly inhibiting or activating 1 or more of the 4 sirtuins tested, more than two-thirds proved to be antiviral with 50% inhibitory concentration <25 μM in a secondary CMV viral growth assay. A medicinal chemistry campaign was performed with >400 molecules synthesized to improve the antiviral activity of a SIRT2 inhibitor identified in the screen (designated FH-003, a 6-{1-[2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-1,3-thiazol-5-yl] ethyl} quinoxaline). Table 3 shows approximately 100-fold improvement in anti-CMV activity with the current lead as well as broad-spectrum activity against multiple DNA and RNA viruses including BK virus, JC virus, influenza A and B, and respiratory syncytial virus. As would be expected for targeting an evolutionally conserved host protein, the broad-spectrum antiviral activity was measured in cells derived from multiple species, including human, monkey, and dog—all demonstrating a therapeutic index >200.

Host targeting predicts favorable antiviral properties, including a high barrier to development of resistance and synergy with known DAAs. Influenza A, a rapidly dividing RNA virus with a higher mutation rate and shorter doubling time than most DNA viruses, was used in these studies [120]. A complete absence of acquired drug resistance was observed after 10 influenza A passages in culture in the presence of the lead SIRT2 inhibitor; in contrast, resistance to the DAA oseltamivir was evident after 2 passages. In addition, HTA plus DAA synergy was demonstrated by testing the interaction between the lead SIRT2 inhibitor and oseltamivir across a wide spectrum of possible drug combinations. The degree of combined viral inhibition ranged from 50% to 90%. Using Chou-Talalay modeling [121], the combination index was 0.68 combination index, or overall combination index < 1, indicating synergy across all relative combinations used.

The mechanism of SIRT2 inhibition on microbe growth is pathogen specific. Infection by *Listeria monocytogenes* induces subcellular localization of SIRT2 from the cytoplasm to the nucleus where epigenetic effects manifest as repression of a significant program of host genes [116]. HBV replication upregulates protein expression of SIRT2 that acts in a feed-forward fashion to increase HBV transcription and replication [113, 114]. Conversely, parainfluenza virus type 3 (HPIV3) reportedly forms viral inclusion bodies dependent on acetylated tubulin for efficient fusion; HDAC6 and SIRT2-mediated

Table 3. Broad-Spectrum Antiviral Effectiveness of Small-Molecule SIRT2 Inhibitors

	DNA Viruses						RNA Viruses											
	HCMV TB40		BK Virus		JC Virus		Influenza A A/ PR/8/1934 H1N1		Influenza A A/ WSN/1933 H1N1 Oseltamivir		Influenza A A/ CA/07/2000 H1N1		Influenza A A/ Perth/16/2009 H3N2		Influenza B B/FL/4/2006 Yamagata		RSV Long Strain	
	Human MRC5		Human HFF		Monkey Cos7		Canine MDCK		Canine MDCK		Human HNBE[c]		Human HNBE[c]		Canine MDCK		Human MRC5	
Compound	IC_{50}[a]	SI[b]	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
FH-003	14	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
cpd1	7.2	…	…	…	…	…	7.9	…	…	…	…	…	…	…	…	…	…	…
cpd2	1.4	>18	0.85	>1592	0.05	>382	9.1	…	…	…	…	…	…	…	…	…	…	…
Lead cpd3	0.45	>56	…	…	…	…	0.76	>12	2.5	>10	**1.2**	>82	**2.1**	>48	1.2	>21	6.7	>3.7
Ganciclovir	1.4	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
Oseltamivir	…	…	…	…	…	…	0.02	>69	9.0	…	…	…	…	…	>25	…	…	…
Ribavirin	…	…	…	…	…	…	…	…	…	…	0.71	>141	0.73	>138	…	…	16.1	>3.1
Cidofovir	…	…	4.4	…	3.8	…	…	…	…	…	…	…	…	…	…	…	…	…

Host-targeted SIRT2 inhibitors FH-003, cpd1, cpd2, cpd3, and direct-acting antivirals tested on indicated virus in indicated cell line at a multiplicity of infection of 0.1.

Abbreviations: HCMV, human cytomegalovirus; HFF, human foreskin fibroblasts; HNBE, human normal bronchial epithelial cekks; IC_{50}, 50% inhibitory concentration; MDCK, Madin-Darby canine kidney cells; RSV, respiratory syncytial virus; SI, selectivity index.

[a]IC_{50} of viral spread given in μM; values in bold indicate 90% inhibitory concentration instead of IC_{50} measurement.

[b]SI indicates the 50% cell cytotoxic concentration/IC_{50}.

[c]Assays performed by the National Institute of Allergy and Infectious Diseases, Division of Microbiology and Infectious Diseases, Resources for Researchers, in vitro testing.

deacetylation of α-tubulin can restrict HPIV3 inclusion body fusion [122]. The mechanism of SIRT2 inhibition on human CMV growth is under investigation. Pathways downstream of SIRT2 posttranslational modifications comprising potential interaction with CMV biology include c-MYC, p53, CDK9, and AKT. The lead SIRT2 inhibitor can potently down-regulate c-MYC protein known to accumulate during CMV infection, presumably to induce efficient glutamine utilization required for viral nucleic acids and lipids [123]. p53 is a direct target of SIRT2 deacetylation [124, 125]. SIRT2 inhibition leads to hyperacetylation and activation of p53, whose transcriptional regulatory activity is known to be substantially inhibited by CMV [126, 127]. SIRT2 also activates CDK9 [128] and AKT kinases [129, 130], known to block the replication [131] or persistence [132], respectively, of CMV.

The current lead in the FH-003 series demonstrates favorable pharmacokinetic properties in mice including 100% oral bioavailability, 1:1 plasma:tissue distribution, a half-life approaching 5 hours, development-amenable in vitro pharmaceutical properties, and manufacturing-scalable synthesis. Experiments are in progress to demonstrate effectiveness in animal models of infection with promising preliminary results. Successful development of HTAs, including sirtuin modulators, will provide an arsenal of agents to use as stand-alone or combination therapies with existing DAAs, foretelling significant patient benefits of improved control of viral load, minimized adverse events, and reduced emergence of viral resistance, as has proven to be the case with HIV combination therapies. Given the intimate relationship between viral infection and host metabolism, broadly effective sirtuin-targeted antivirals may also address the unmet need of infection by pandemic viruses yet to be identified.

In conclusion, human CMV infections remain a considerable cause of morbidity and mortality. As the number of immunosuppressed individuals increases, with organ transplantation becoming more common, together with the mandate of therapy for congenitally infected infants, improved outcome is essential [22]. Newer approaches should explore combination therapies.

Notes

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