Emerging Concepts in Congenital Cytomegalovirus

Megan H. Pesch, MD, MS,[a] Mark R. Schleiss, MD[b]

Over a century of research has focused on improving our understanding of congenital cytomegalovirus (cCMV), yet it remains the most common congenital infection in the United States, affecting 3 to 6 per 1000 live born infants each year. Pregnancies affected by cCMV are at a heightened risk of spontaneous abortion and intrauterine fetal demise. Neonates born with cCMV are also at substantial risk for long-term neurodevelopmental sequelae and disability, including sensorineural hearing loss, even those born without clinically apparent disease. Considerable progress has been made in recent years in study of the epidemiology and transmission of cCMV, developing better diagnostic strategies, implementing newborn screening programs, improving therapeutics, and launching vaccine trials. In this article, we review recent developments in the understanding of the virology and immunobiology of cytomegalovirus. We further discuss how this knowledge informs our understanding of the pathophysiology of cCMV and directs strategies aimed at improving outcomes and quality of life for congenitally infected children. We also provide an update on the epidemiology of cCMV in the United States, evolving scientific understanding of maternal-fetal transmission, enhanced screening approaches, and recognition of neonatal and long-term sequelae. Finally, we review the current landscape of pediatric cCMV research and provide recommendations for novel and high-priority areas for future investigation.

abstract

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STATE-OF-THE-ART-REVIEW

TABLE 1 On-Going and Recently Completed Studies of Congenital Cytomegalovirus Related Therapeutics, Prevention, and Vaccines

Clinical Trial Number, Principal Investigator, Institution or Sponsor	Phase	Intervention	Primary Objective	Population
Antiviral medication for infants with cCMV				
NCT03301415 National Institute of Allergy and Infectious Diseases[115]	Phase 2	Valganciclovir, 16 mg/kg per dose provided orally twice daily for 4 mo	To evaluate valganciclovir as a treatment to prevent development of SNHL in infants with asymptomatic cCMV infection	229 newborns with asymptomatic cCMV without baseline SNHL
NCT03107871 Albert Park, University of Utah, National Institute of Deafness and Other Communication Disorders, Genentech, Inc.[116]	Phase 2	Valganciclovir, 16 mg/kg per dose or placebo provided orally twice daily for 6 mo	To determine if valganciclovir treatment reduces the mean slope of total hearing thresholds over the 20 mo after randomization compared with placebo in cCMV-infected asymptomatic infants with isolated hearing loss	52 infants with asymptomatic cCMV and isolated SNHL
NCT02005822 Ann C.T.M. Vossen, Leiden University Medical Center, Netherlands[117]	Phase 3	Valganciclovir, 16 mg/kg per dose provided orally twice daily for 6 wk	To investigate whether early treatment with oral valganciclovir of infants with both congenital cytomegalovirus infection and sensorineural hearing loss can prevent progression of hearing loss	37 infants with cCMV and SNHL
Maternal immunoglobulin to prevent vertical transmission				
NCT05170269 Biotest AG	Phase 3	Cytotect CP Biotest (BT097), 200 U/kg IV weekly every 2 wk until at least GW 17	To demonstrate efficacy and safety of Cytotect CP Biotest in preventing maternal-fetal transmission of CMV	80 pregnant women with primary CMV infection
Maternal behavioral intervention to prevent CMV infection				
NCT04615715 Karen Fowler, University of Alabama at Birmingham[49]	NA	Prenatal clinic-based CMV risk-reduction behavioral intervention versus control	To evaluate whether a brief prenatal clinic-based CMV risk-reduction behavioral intervention will prevent maternal CMV infections during pregnancy in women	840 pregnant women
Antiviral medication for congenitally infected fetuses				
NCT04732260 Yves Ville, Assistance Publique - Hôpitaux de Paris, France[118]	NA	1 tablet of Letermovir (240 mg or 480 mg /d) for 3 d	To measure the Letermovir transplacental transfer in the second trimester and its accumulation in the amniotic fluid and the placenta in the second trimester	10 pregnant women undergoing termination of pregnancy for fetal abnormality
CMV vaccine trials				
NCT03486834 Merk Sharp and Dohme Corp.[119]	Phase 2	Vaccine V160 vs placebo	To evaluate the safety, tolerability, and efficacy of the CMV vaccine (V160) and whether administration of a 3-dose regimen reduce the incidence of primary CMV infection compared with placebo	2200 healthy seronegative women aged 16 to 35 y with direct exposure to young children
NCT05089630 GlaxoSmithKline[120]	Phases 1 and 2	Recombinant protein subunit vaccine	To assess the safety, reactogenicity, and immunogenicity of the candidate CMV recombinant protein subunit, regardless of baseline CMV sero-status	320 healthy adults 18 to 50 y of age
NCT05085366 ModernaTX, Inc.[121]	Phase 3	mRNA-1647 vaccine versus placebo	To evaluate the efficacy of mRNA 1647 vaccine in CMV-seronegative female participants and to evaluate the safety and reactogenicity of mRNA-1647 vaccine in all participants	6900 healthy seronegative participants aged ≥20 y old with direct exposure to at least 1 child ≤ 5 y old

cCMV, congenital cytomegalovirus; CMV, cytomegalovirus; GW, gestational week; SNHL, sensorineural hearing loss

Ever since the first published recognition of congenital cytomegalovirus (cCMV) disease in a stillborn fetus in 1881, over 140 years of research has focused on improving our understanding of this pathogen toward the goal of mitigating its impact on human health.[1] Cytomegalovirus (CMV) is the most common congenital infection in the United States, and a leading cause of neurodevelopmental disabilities, including sensorineural hearing loss (SNHL).[2] Despite the impact that cCMV has on newborn health, awareness among healthcare providers is suboptimal.[3] There is a need to translate novel observations about the basic science of CMV virology and the host immune response to clinical practice and public policy. With cCMV newborn screening programs being implemented in several states, it is likely that pediatricians will increasingly encounter this infection in clinical practice.[4–6] Moreover, several CMV vaccines are moving forward in clinical trials (Table 1),[7] and if successful, the licensure of a vaccine will, in turn, drive increased awareness and raise questions of the impact of cCMV infection on pediatric practice. Significant advances in the understanding of pathophysiology, epidemiology, diagnostic approaches, treatment, and long-term outcomes of cCMV have been made in the last 3 to 5 years. The burden of cCMV is disproportionately incurred by low- and middle- income nations, an important factor in public policy discussion, including newborn screening.[8,9] The aim of this paper is to review the state-of-the-science of cCMV in the United States, highlighting recent developments that will inform future clinical management and research.

CMV GENOME, REPLICATION, AND GENE EXPRESSION: ROLE IN VIRAL PATHOGENESIS

CMV is a member of the *Herpesviridae* family of viruses, and has a large, double-stranded DNA genome, with a total coding content of >230 kbp of sequence.[10] CMV genomes consist of unique long and unique short segments, each bracketed by terminal repeats[10]; 4 different isomeric configurations of these segments are therefore possible in any given virus particle (Fig 1). Hence, some viral gene products are diploid, with duplicate copies in both the terminal repeat and internal repeat regions of the unique long or short regions of the 2 genome segments, respectively. From a clinical perspective, an understanding of these mechanisms of strain diversity informs the phenomenon of reinfection.[11] It has become increasingly clear that reinfection with novel variants can occur in women with prior prepregnancy immunity, and that such strain variants can be transmitted to the fetus.[12] The rapid generation of novel CMV strains has also posed a challenge for vaccine and antiviral medication development given the rapidly-evolving nature of the "target."[13]

The CMV genome includes many genes directed at evading, blocking, impairing, and modulating host viral defenses.[14–16] CMV envelope glycoproteins, structural proteins, and nonstructural proteins (Table 2) play important roles in the virus's carefully choreographed host-immune evasion phenotype. These gene products function by interfering with the generation of CD4+ and CD8+ T-cell responses, impeding natural killer cell clearance of infection, altering host cytokine and chemokine responses, and blocking innate immune responses mediated by interferons.[14] Together, CMV immune evasion genes function to subvert protective immunity and likely play a key role in promoting life-long infection, periodic reactivation, and reinfection with novel strain variants.

Advances in the molecular characterization of patterns of host gene expression have facilitated study of the host transcriptome as a biomarker for gauging the risk of cCMV sequelae.[17] In one such study, blood transcriptional profiles were compared in infants with symptomatic and asymptomatic cCMV infection. A 16-gene host genetic "signature" was identified, which was associated with the development of SNHL in both infant categories.[17] Understanding of the components of viral transcriptomics that contribute to outcomes may facilitate future clinical management by identifying infants that need more detailed audiological or neurodevelopmental follow-up or may be more likely to benefit from antiviral therapy. Similarly, viral strain variation has been shown to impact the pathogenesis of CMV-induced injury.[18,19] Although not yet available for bedside diagnostics, development of viral genotypic assays to identify "high-risk" variants may prove to be a useful adjunct to identifying reinfections of previously seropositive women during pregnancy, and to direct clinical management of infants with cCMV.

EPIDEMIOLOGY, TRANSMISSION, AND PRESENTATION OF CONGENITAL INFECTION

A Review of the Basics

After an initial or primary infection, CMV establishes a lifelong infection in the host. Although the virus becomes quiescent, establishing latency in hematopoietic progenitor cells,[20] reactivation occurs throughout the lifetime of the host under conditions of stress, intercurrent illness, and immune suppression.[21] Reactivation of latent CMV is thought to rarely cause disease in immunocompetent hosts but can lead to heightened morbidity and mortality in immunocompromised individuals.[20]
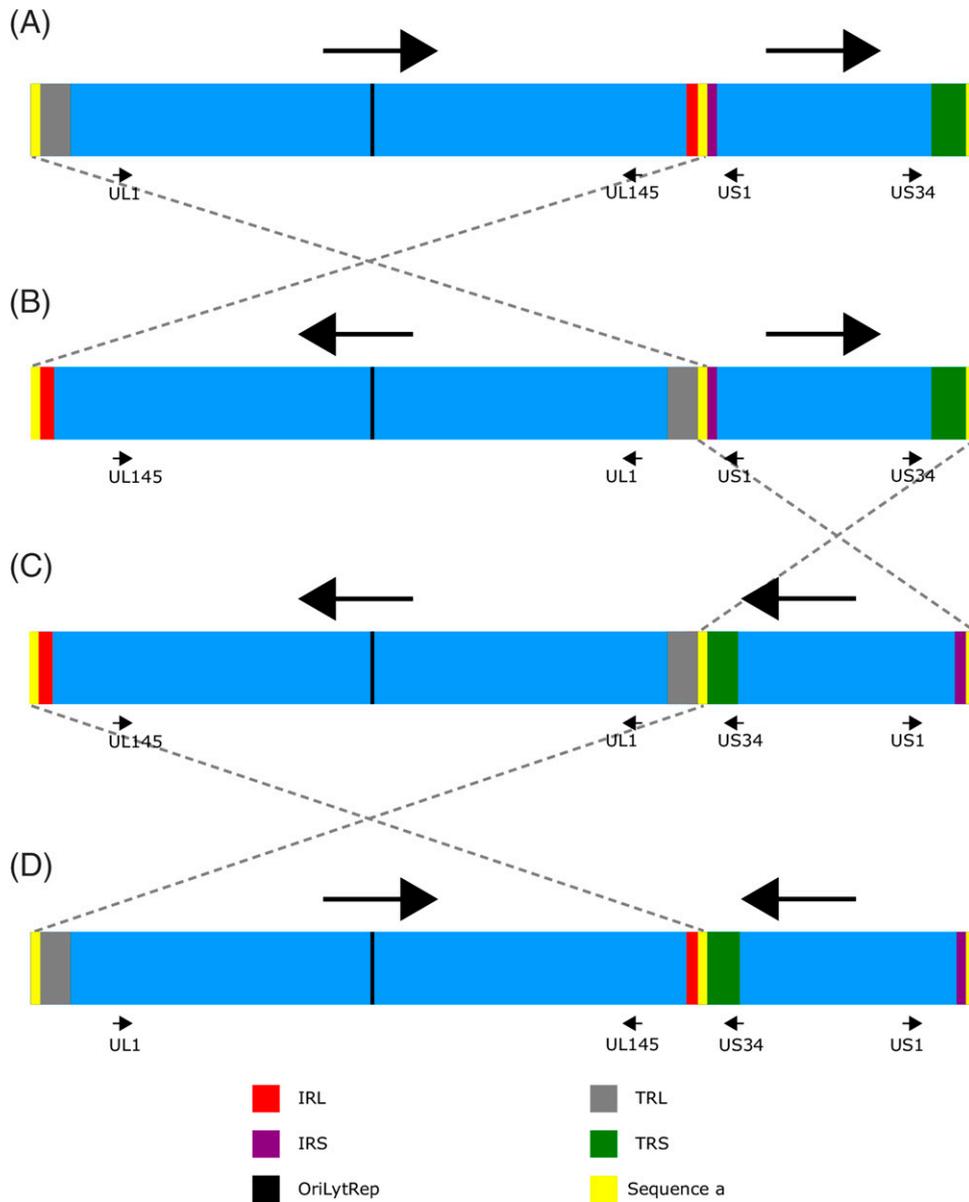
FIGURE 1

Structure and isomerization of HCMV genome (Marti-Carreras and Maes, 2019, PMID 30604286). Representation of the HCMV genome (not on scale) with its 4 possible isomers (panels A–D). In panel A, the orientation of the unique long region (UL) and unique short region (US) are shown, based on UL- and US- orientation of a HCMV wild-type strain known as the "Merlin" strain (GenBank AY446894). During replication, the 2 unique regions can pair in any of 4 different isomeric configurations. Panels B through D correspond to the other 3 possible isomeric orientations. The genome also contains diploid repeat sequences, either internal to the isomeric form of a given genome (inverted repeats, or IR regions) or the termini of the mature genome (terminal repeats, or TR regions). These exist in both the long repeat regions (L) and for the short repeat regions (S). Genome regions that are characteristic for HCMV hence consist of: IRL, IRS, TRL, and TRS. The lytic origin of replication, known as oriLyt, is indicated. These repeat regions, the origin of replication repetitive sequence (OriLytRep), and another series of highly repetitive sequence, known as the "a" sequences (a′) are colored in red, purple, gray, green, black and yellow, respectively. Small black arrows correspond to the direction of selected open reading frames, including UL1, UL145, US1 and US34, which help to illustrate the orientation of the unique regions (big black arrow) between the different isomers. Dashed gray lines connect the specific a′ sequences that contributed to the isomerization. The isomerization of the HCMV genome during replication is a factor that helps to contribute to strain variation (see text for details). (Reprinted with permission from Marti-Carreras and Maes. Human cytomegalovirus genomics and transcriptomics through the lens of next-generation sequencing: revision and future challenges. *Virus Genes.* 2019;55(2)138–164.)

TABLE 2 Cytomegalovirus-Encoded Immunogens Serving as Candidate Targets in Development for Inclusion in a Cytomegalovirus Vaccine

CMV Gene Product		Rationale for Inclusion
Glycoproteins(outer lipid bilayer envelope)	Glycoprotein B (gB)	This glycoprotein is necessary for CMV entry into all cell types. gB is also highly immunogenic and is a major target of neutralizing antibodies and cytotoxic T-lymphocytes. The gB target has been a cornerstone of CMV vaccine development programs; demonstrated efficacy as subunit vaccine in clinical trials
	gH/gL glycoprotein complex	Important target of neutralizing antibodies: target of cytotoxic T-lymphocytes
	gH/gL/UL128/UL130/UL131 complex (pentamer of CMV proteins)	UL128, UL130 and UL131 are CMV genes that encode 3 small proteins involved in signaling and bind to glycoprotein gH/gL creating the pentameric complex of gH/gL/UL128/UL130/UL131 on the CMV viral envelope. The complex mediates infection at epithelial and endothelial cell surfaces. This pentamer is the target of neutralizing host antibodies, which block infection at the surface and is the focus of clinical vaccine trials (in combination with gB)
	gH/gL/g0 complex (trimer of CMV proteins)	This viral envelope trimer facilitates CMV infection of fibroblasts and is the target of host neutralizing antibodies which block cell entry and fusion
Structural proteins (tegument layer)	Phosphoprotein 65 (pp65)	Tegument protein responsible for evading the host immune response and is a target of cytotoxic T-lymphocytes and nonneutralizing antibody responses; has been evaluated (in combination with gB) in clinical trials
Nonstructural proteins	Immediate early protein-1	This protein plays a role in modulating the host cell environment for expression of viral genes. Important target of CD8+ cytotoxic T-lymphocytes and nonneutralizing antibody responses; has been evaluated (alone or in combination with gB and UL83 [pp65]) in clinical trials

CMV, cytomegalovirus; gB, glycoprotein B.

Prior infection with CMV and circulating antibodies do provide some protection, however, lowering the risk of vertical transmission and severity of manifestations of congenital infections.[22] Because of the high prevalence of CMV in the US population, it is estimated that most infants with cCMV are born to women with preexisting exposure to the virus.[23]

As an overview, most infants (85% to 90%) are born with clinically inapparent infections, and the remaining 10% to 15% are born with symptomatic disease (visible signs, laboratory abnormalities, and/ or intracranial abnormalities).[24–26] The absence of visible signs at birth is not always associated with an asymptomatic infection (defined as having no signs, laboratory, or intracranial abnormalities at birth).[27] Some well-appearing infants with cCMV may still have intracranial involvement and thus be categorized as symptomatic.[28] Less than 5% of infants with cCMV present with the classic "blueberry muffin" triad (small for gestational age, direct hyperbilirubinemia, and a petechial rash). Infants with symptomatic disease are at heightened risk of neonatal death and of long-term neurodevelopmental sequelae.[25,27,29,30] SNHL may be present at birth or develop over childhood in those with asymptomatic or symptomatic infections.[26,31] Infants with isolated SNHL are generally considered as having an asymptomatic infection, although some experts argue that SNHL is a marker of central nervous system (CNS) involvement and should be treated similarly to symptomatic disease.[27]

Recent Findings in cCMV Epidemiology

Congenital CMV occurs in 3 to 6 infants in every 1000 live births in the United States each year.[32] Studies of cCMV prevalence in the United States have identified disparities among marginalized communities. A study of roughly 100 000 neonates screened at birth found cCMV prevalence to be highest among African American infants (9.5 per 1000 live births), followed by multiracial infants (7.8 per 1000 live births), with the lowest prevalence among non- Hispanic White infants (2.7 per 1000 live births) and Asian infants (1.0 per 1000 live births).[32] Even after adjusting for socioeconomic status and maternal age, African American infants were nearly twice as likely to have cCMV compared with non-Hispanic White infants.[32,33] Furthermore, Native Americans and African Americans have also been found to be roughly 2 times more likely, respectively, to die of cCMV than non-Hispanic White infants.[30] Social determinants of health and systemic racism may contribute to high rates of CMV seropositivity among pregnant women living in urban, low-income areas, particularly among those of racially marginalized groups.[34]

Attention has recently been drawn to the role cCMV plays in intrauterine fetal demise and miscarriage. A large multisite US study examining intrauterine fetal demise due to infectious etiologies identified cCMV as the likely cause in 8% of cases.[35] This builds on prior work from Australia that identified CMV on fetal autopsy in 15% of stillbirths at >20 weeks gestational age.[36] A meta-analysis

found maternal CMV infection during pregnancy to be a risk factor for spontaneous abortion (OR 1.61, 95% CI 1.14%–2.27%) and intrauterine fetal demise (5.74, 95% CI 2.04%–16.12%).[37]

Transmission

CMV is transmitted horizontally in bodily fluids, including urine, saliva, vaginal secretions, semen, breast milk, and plasma. Women with more than 1 child and those who work with children are at the highest risk of having an infant with cCMV, as close exposure to body fluids from young children increases the likelihood of horizontal transmission.[38] Toddler and preschool-aged children shed CMV in their saliva and urine for months after an infection, long after their symptoms have resolved.[39,40] Behaviors to lower an individual's risk of horizontal transmission include hand hygiene after changing diapers, not sharing food or utensils with young children, and avoiding kissing young children on the lips.[24,41] Pediatricians are uniquely positioned to educate mothers about the risks of and prevention strategies against acquisition of CMV from their toddlers, which in turn may be of benefit for future pregnancies.[42–44] Prenatal education about CMV prevention behaviors is standard of care in several countries.[45–48] Furthermore, such behavioral interventions have been found to be acceptable to women and associated with lowered rates of seroconversion in pregnancy.[41,42,47] An on-going randomized controlled trial (RCT) of prenatal education, versus standard of care, may provide even more supportive evidence (Table 1).[49] Early data suggest that the prevalence of cCMV dropped dramatically in 2020 as compared with 2019, coinciding with the COVID-19 pandemic.[50] It may be that increased hand hygiene and the closure of childcare centers

resulted in fewer opportunities for horizontal transmission and thus, fewer cases of cCMV.[50]

Mechanisms of and protective factors against vertical transmission are an area of active inquiry. A recent meta-analysis of twin pregnancies affected by CMV found an elevated rate of vertical transmission (59% vs 31% in singleton pregnancies) and cCMV discordance in 50% of twin pregnancies (21 of 42).[51] cCMV concordance occurred in 95% of monozygotic twin pairs and 38% of dizygotic twin pairs, resulting in an estimated hereditability of 94%.[51] These findings point toward possible placental differences or genetic factors conferring increased susceptibility to vertical transmission of CMV.[51] Genetic variations in maternal toll-like receptor single nucleotide repeats have been implicated both in increased[52] and decreased rates of CMV infection during pregnancy.[53]

Prenatal treatment to lessen the risk of vertical CMV transmission have been the focus of some small studies and 2 recent RCTs.[54–57] Hughes et al sought to evaluate whether cytomegalovirus immune globulin lowered the risk of vertical transmission but the trial was stopped early because of futility and safety concerns.[55] A randomized double-blind placebo-controlled trial of valacyclovir in pregnant women with primary CMV infections in the first trimester found lower odds of vertical transmission in the treatment group.[57] The potential impact of in-utero antiviral treatment in ameliorating sequelae in CMV-infected fetuses has also been studied. An open-label trial of valacyclovir in women pregnant with mildly symptomatic cCMV-infected fetuses found that a greater proportion delivered infants with asymptomatic infections (82%) as

compared with rates of untreated controls from the literature (43%).[58]

CCMV SCREENING, DIAGNOSIS, AND EVALUATION

Screening

Newborn screening for cCMV has become more common over the last several years, with the goal of early identification of and intervention for affected infants. Hearing-targeted cCMV screenings target testing only to infants who fail their newborn hearing screening, which is nearly eightfold more common in infants with cCMV (versus those without cCMV),[59] unlike a universal screening approach in which all neonates are tested. However, Fowler et al found that only 57% of infants with cCMV associated hearing-loss in the neonatal period were identified by hearing-targeted cCMV screening protocols, which is arguably suboptimal when compared with universal screening.[59] Seven states now have legislative mandates for hearing-targeted cCMV screening (Illinois, Iowa, Connecticut, New York, Utah, and Virginia), and 1 state (Minnesota) recently passed a universal screening bill.[60] There remains an active debate about the cost-effectiveness of cCMV screening programs, as well as the economic burden of cCMV.[61]

Studies support parental acceptance of and a desire for cCMV newborn screening.[62,63] Tastad et al found that 96% of women supported newborn screening once they were educated about the public health significance of the topic.[62] In a survey study of 3922 participants, most parents strongly or somewhat agreed that they would want to have their newborn tested for cCMV, even if it was not performed routinely (84%), if they had to pay out-of-pocket for the test (87%), or if CMV-

related problems never developed in their child (84%).[63]

Diagnostic Testing

Newer point-of-care testing platforms for cCMV have allowed for the growth of cCMV screening programs.[64] For context, screening or testing for cCMV may be performed using nucleic acid amplification testing (eg, polymerase chain reaction [PCR] using dried blood spot [DBS], saliva, or urine). The timing of screening and confirmatory testing for cCMV is important and must be performed on a fresh sample (eg, urine or saliva) before 21 days of age, after which it is challenging to distinguish a congenital infection from a postnatal exposure.[27]

If saliva-based screening is employed, strong consideration should be provided to confirming positive saliva results with a urine PCR because of the substantial risk of a false positive result attributable to the presence of "carry-over" CMV DNA in saliva due to the presence of viral genome in breast milk from a seropositive nursing mother. The risk of false positive results can be minimized by collecting the specimen >1 hour after breastmilk consumption.[65] Retrospective cCMV testing can be performed on saved samples collected at birth, such as cord blood, umbilical stump, or DBS remnants. Recent work found the sensitivity of DBS CMV PCR in 2 laboratories to be 74% and 77%, with a 2-primer approach yielding a sensitivity of 86%, suggesting that newborn screening programs focused on DBS PCR may need to use multiprimer and multiplex approaches to maximize sensitivity.[66] Whereas somewhat higher than the 30% to 40% sensitivity reported a decade ago, present day DBS PCR sensitivity remains relatively low as compared with the near 99% sensitivity of saliva and urine assays.[64,66] This concern may be mitigated by the fact that DBS collection is a standard practice in newborns to screen for a variety of genetic and metabolic diseases; thus, the infrastructure exists to rapidly incorporate DBS screening into standard clinical care, greatly reducing the overall cost of such programs.

Presentation and Sequelae

The presentation of cCMV varies widely across the spectrum of disease severity, although it is most commonly clinically inapparent.[67] A detailed review of the clinical signs and recommendations for management are beyond the scope of this paper; the following sources for reference on those subjects are recommended.[24,27] Once a diagnosis of cCMV is confirmed, additional diagnostic evaluation is critical in determining the presence of sequelae and the infection severity, which dictates further treatment and monitoring. Below, a summary of recent research surrounding the most common sequelae of cCMV is presented:

Sensorineural Hearing Loss

All infants with cCMV are considered to be at high risk for SNHL and warrant diagnostic testing and close audiologic follow-up, according to the most recent Joint Committee on Infant Hearing guidelines.[68] SNHL occurs in 15% to 25% of all children with cCMV over the course of childhood.[31,69] The development of SNHL is associated with first-trimester (versus third) infection, symptomatic disease, and intracranial involvement.[69,70] Unique to cCMV-related SNHL is the frequent progression and fluctuation of hearing thresholds, which occurs in roughly ~45% of cases.[70,71] Work by Lanzieri et al reported SNHL at age 18 years in 25% of children with asymptomatic cCMV, 72% of whom had a delayed onset of SNHL.[72]

Ophthalmologic Manifestations

Symptomatic cCMV has been associated with several ophthalmologic manifestations, which can contribute to abnormal visual acuity and visual impairment over childhood and early adolescence. Children with symptomatic cCMV are more likely to have moderate or severe visual impairment compared with those with asymptomatic infection.[73] Overall, the presence of clinical symptoms of cCMV at birth, SNHL, and microcephaly have been found to be predictors of severe visual impairment.[73] Of note, children with asymptomatic infection and those with symptomatic disease with no evidence of retinal or posterior pathway involvement in early life have been found to have visual acuity comparable to healthy controls in adulthood.[74] Other work has also found little evidence of ocular sequelae in those with asymptomatic infections.[74,75] Yearly ophthalmologic evaluations are recommended for those with symptomatic cCMV disease.[73]

Intracranial Abnormalities

Increased attention has been paid to the broad spectrum of neuroimaging abnormalities seen in cCMV, moving away from a dichotomous characterization of "normal" versus "abnormal." Nonbinary severity rating scales covering the range of potential intracranial abnormalities may lead to more accurate neurodevelopmental prognoses.[76–81] A recent study found a MRI severity score to be a better predictor of adverse neurologic sequelae than the presence of symptoms at birth.[82] Expert cCMV clinical guidelines recommend all infants receive a cranial ultrasound (CUS), followed by a MRI, if abnormalities are seen, and antiviral treatment of those
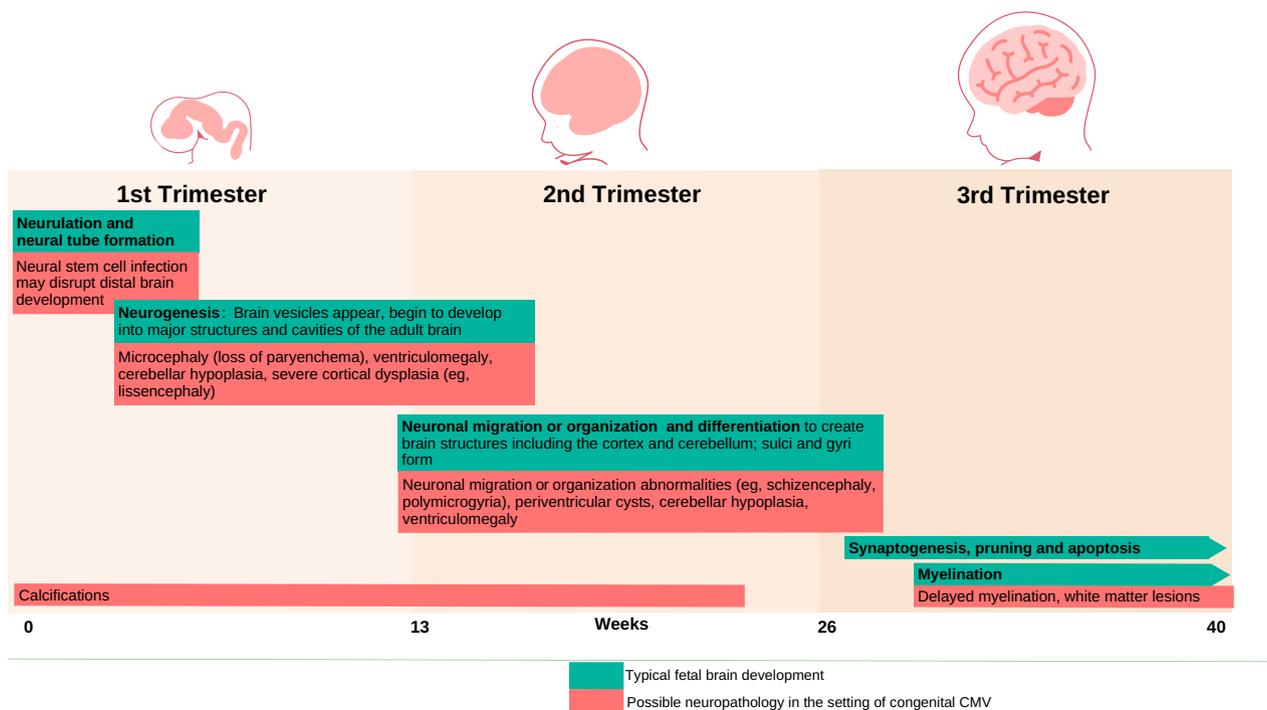
FIGURE 2
Typical fetal brain development and resultant congenital cytomegalovirus-associated neuropathology by timing of maternal infection and vertical transmission. Processes of fetal neurodevelopment distal to timing of fetal infection may be affected by CMV-associated placental injury, inflammatory response, and fetal central nervous system infection.[91,113,114]

with CNS involvement.[27,48] Recent studies have highlighted the complementary nature of CUS and MRI for evaluating cCMV CNS involvement.[83,84] Work from the Netherlands found that 20% (93 of 480) of infants with cCMV with normal CUS findings had abnormalities found on MRI, 37% of whom were categorized as severely symptomatic based on MRI alone.[84] Vande Walle et al reported MRI abnormalities in 33% to 59% of infants with clinically inapparent cCMV in their cohort.[85] The clinical significance of subtle isolated MRI findings remains unclear.[86] Whereas severe lesions, such as polymicrogyria or lissencephaly, are likely irreversible regardless of antiviral treatment, some have proposed that infants with isolated white matter abnormalities may be more susceptible to treatment,[87] but this remains to be proven.

Longitudinal studies of the neurodevelopmental outcomes of infants with isolated subtle MRI findings are needed to inform clinical guidance around neuroimaging and antiviral treatment.

Emerging Concepts in cCMV Neuropathogenesis

CMV is a neurotropic virus, readily crossing the fetal blood-brain barrier and infecting and replicating in brain-resident cells, including astrocytes, neurons, and microglia.[88] The lesions caused by cCMV are best understood according to the timing of infection in pregnancy and corresponding stages of fetal brain development (Fig 2). Earlier trimester infections result in the most severe neuropathology because CMV disruption of fetal cortical development from aberrant neuronal migration, formation, and organization results in

polymicrogyria, cerebellar hypoplasia, and more rarely, lissencephaly.[23,89,90] Infections later in pregnancy after the architecture of the fetal brain has developed may result in inflammation, causing white matter lesions and cysts.[91]

Potential molecular mechanisms of CMV-dysregulated cortical development have been explored. One study evaluated CMV-infected neural stem cells, demonstrating increased expression of the gene *PAFAH1B1*, encoding LIS1 (lissencephaly-1).[92] LIS1 is a component of the platelet activating factor acetyl-hydrolase complex, which is important in directing neuronal migration. Increased immunostaining for LIS1 in brains of congenitally infected fetuses, but not controls, has been found, strengthening the hypothesis that dysregulation of *PAFAH1B1* provides at least one explanation for cCMV

brain injury. Other molecular mechanisms may also mediate fetal CNS injury. CMV has been found to induce downregulation of the gene, *nidogen 1* (NID1), a basement membrane protein important for neuronal migration, regulating Schwann cell proliferation, migration, and myelin production.[93] It is hypothesized that virus-induced downregulation of NID1 might offer CMV a means of increased distribution throughout the host.[93]

The host inflammatory response can also contribute to CMV neuropathy. Infected microglia and astrocytes become active overproducers of cytokines, leading to a wide-spread proinflammatory response in the developing fetal brain.[94] Inflammatory infiltrates have been found in close approximation to CMV-positive neurons, suggesting a significant component of immune-mediated injury.[95,96] Similarly, CMV infection and inflammatory infiltrates have been found throughout the structures of the inner ear, including the stria vascularis, which plays a critical role in potassium and ion homeostasis in the cochlea.[97] It has been proposed that CMV-mediated dysregulation of such homeostatic mechanisms could, by altering the generation of endocochlear action potentials, produce SNHL.[98]

INTERVENTIONS AND LONG-TERM OUTCOMES

Interventions

Interventions and treatment of cCMV largely consist of standard management of sequelae (eg, antiepileptic drugs for seizures, physical therapy for gross motor delays), which are nonspecific to cCMV. Monitoring for audiologic, visual, and developmental sequelae is also a form of intervention. Below, recent findings in the field specific

to the treatment of cCMV are highlighted.

Antiviral Medication

In 2015, Kimberlin et al published the results of a RCT of a 6-month versus 6-week course of oral valganciclovir (the prodrug of IV ganciclovir) for infants with symptomatic cCMV, finding modest improvement in hearing and neurodevelopmental outcomes at 24-months in the group treated for 6 months.[99] Guidelines recommend valganciclovir treatment of infants with moderate-to-severe symptomatic cCMV. The possible benefits of therapy must be balanced with the risks of adverse events, particularly neutropenia.[100] The safety and efficacy of valganciclovir use in less severely affected infants has not been established; results of ongoing studies are much anticipated (Table 1).

Advances in Cochlear Implantation

For infants with severe-to-profound SNHL, cochlear implantation can now be performed before 9 months-of-age.[101] Early cochlear implantation and language exposure has been associated with improved spoken language outcomes in children with SNHL.[102] Children deafened by cCMV have been found to have adequate access to sound for speech production, but their spoken language scores tend to be lower than children deafened by other etiologies (eg, Connexin 26 mutations), which is likely related to other cCMV comorbidities.[103]

Long-Term Outcomes

Asymptomatic cCMV

Most infants born with asymptomatic cCMV have been found to have neurodevelopmental outcomes in the typical range,[26] though recent work has highlighted some areas of risk. In 1 of the only longitudinal studies of children with asymptomatic cCMV followed into

adolescence, Lopez et al found that those with SNHL (but not those with typical hearing) had lower measures of verbal skills as compared with hearing controls, which was likely attributable to hearing loss.[104] There were no differences in nonverbal intelligence or academic achievement in math and reading between adolescents with cCMV-associated SNHL and unaffected sibling controls.[104] Pinninti et al identified a high prevalence of vestibular (45%), gaze (46%), and balance (30%) disorders in children with asymptomatic cCMV with and without hearing loss.[105]

Symptomatic cCMV is associated with greater risk of long-term disabilities. Lanzieri et al followed 76 children with symptomatic cCMV through median age 13-years, finding 43% with intellectual disability, 74% with SNHL, 27% with visual impairment, and 42% with both intellectual disability and SNHL.[106] Congenital CMV is also thought to be prevalent in children with cerebral palsy, although there does not seem to be a particular phenotype of cerebral palsy associated with cCMV.[107] Some preliminary studies point to a possible correlation between symptomatic cCMV and autism spectrum disorder; additional research is needed to establish causation.[108]

FUTURE DIRECTIONS

Progress Toward a CMV Vaccine

Several candidate vaccines against CMV are currently in development (Table 1).[7] Whereas the ideal population for a CMV vaccine would be young children who would carry immunity into adulthood, similar to the measles, mumps, and rubella vaccine, developers are first focusing on women of childbearing age. A vaccine in this population may serve to reduce horizontal infection to the
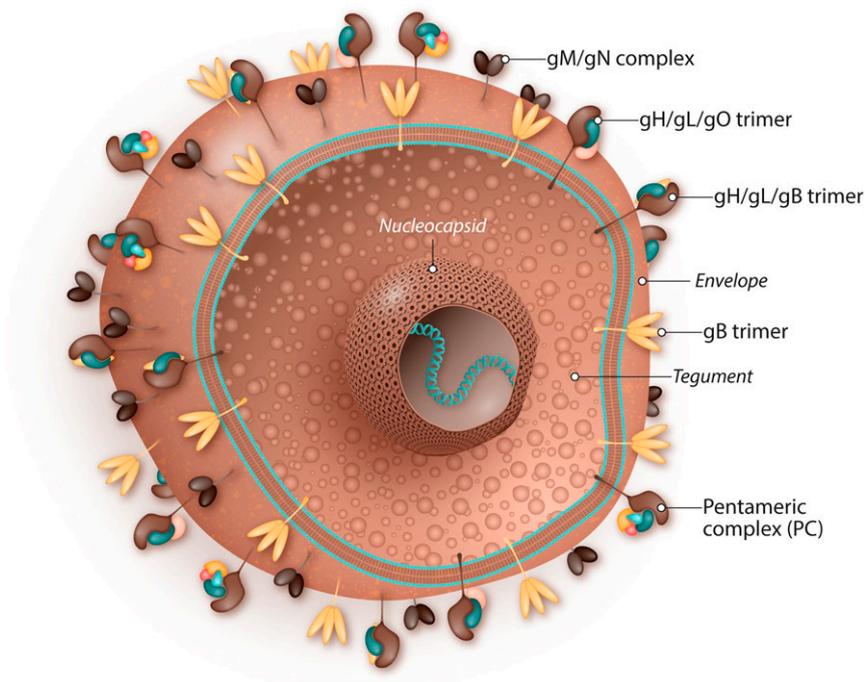
FIGURE 3
CMV virion demonstrating key envelope and glycoprotein complexes important in protective immunity that stand as potential subunit vaccine candidates. Viral envelope, tegument, and nucleocapsid are as indicated. Glycoprotein complexes are labeled; see Table 1 for additional details. gB, glycoprotein B; gH, glycoprotein H; gL, glycoprotein L; gM, glycoprotein M, gN, glycoprotein N; gO, glycoprotein O.

mother, and thereby reduce vertical transmission to the fetus. Prospective studies of long-term immunity conferred by a CMV vaccine may provide support for childhood immunization. Strain variation has posed a challenge for vaccine development; although the molecular mechanisms leading to novel variants are different for CMV (a DNA virus) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (a RNA virus), strain variation complicates CMV vaccine design, just as it does for COVID-19 immunization strategies.[109]

Subunit vaccines against CMV have focused on the 3 distinct regions of the CMV virus particle: the capsid, the tegument layer, and the envelope (Fig 3 and Table 2). The icosahedral capsid consists of 162 capsomere subunits that enclose the viral genome.[110] In the mature virus, the capsid is surrounded by the tegument, a layer containing a 65-kilodalton phosphoprotein that is a major T-cell target referred to as pp65.[111] Finally, surrounding the tegument is the outer lipid bilayer envelope, containing virally encoded glycoproteins.[112] The glycoprotein immunogens currently being explored in clinical trials as subunit vaccine candidates are outlined in detail in Table 1.

High Priority Areas for cCMV Research

In addition to vaccine development, other areas of high priority in cCMV

research include DBS PCR assay improvement, therapies to reduce vertical transmission, as well as safe and effective antiviral medications for all neonates with cCMV. In terms of clinical management, movement away from the binary categorization of cCMV as asymptomatic versus symptomatic toward a spectrum of disease may lead to more nuanced studies and treatment recommendations. Research is needed to improve our understanding of developmental outcomes and trajectories, based not only on presentation at birth, but also services and supports in childhood that may ameliorate outcomes. Identification of biomarkers of disease severity will aid in developing more nuanced studies and ultimately, interventions. Lastly, better elucidating the economic burden of cCMV disease, as well as costs of screening, treatment, and prevention programs is of high priority.[61] These studies should include measures of direct healthcare costs, but also indirect costs, such as caregiver burden and quality of life.[61] A better understanding of the societal impact of cCMV is important for public health prioritization to reduce pregnancy loss, childhood death, and disability from this common yet often inapparent virus.[113,114]

ABBREVIATIONS

cCMV: congenital cytomegalovirus
CMV: cytomegalovirus
DBS: dried blood spot
PCR: polymerase chain reaction
SNHL: sensorineural hearing loss

REFERENCES

1. Ho M. The history of cytomegalovirus and its diseases. *Med Microbiol Immunol (Berl)*. 2008;197(2):65–73

2. Macedo-da-Silva J, Marinho CRF, Palmisano G, Rosa-Fernandes L. Lights and shadows of TORCH infection proteomics. *Genes (Basel)*. 2020;11(8):894

3. Fellah T, Sibiude J, Vauloup-Fellous C, et al. Evolution of awareness and knowledge of congenital cytomegalovirus infection among health care providers in France between 2011 and 2018. *J Clin Virol*. 2020;129:104335

4. Schleiss MR. Congenital cytomegalovirus: impact on child health. *Contemp Pediatr*. 2018;35(7):16–24

5. Dollard SC, Dreon M, Hernandez-Alvarado N, et al. Sensitivity of dried blood spot testing for detection of congenital cytomegalovirus infection. *JAMA Pediatr*. 2021;175(3):e205441

6. Ronchi A, Shimamura M, Malhotra PS, Sánchez PJ. Encouraging postnatal cytomegalovirus (CMV) screening: the time is NOW for universal screening! *Expert Rev Anti Infect Ther*. 2017;15(5):417–419

7. Plotkin SA, Wang D, Oualim A, et al. The status of vaccine development against the human cytomegalovirus. *J Infect Dis*. 2020;221(suppl 1):S113–S122

8. Cannon MJ, Davis KF. Washing our hands of the congenital cytomegalovirus disease epidemic. *BMC Public Health*. 2005;5:70

9. Ssentongo P, Hehnly C, Birungi P, et al. Congenital cytomegalovirus infection burden and epidemiologic risk factors in countries with universal screening: a systematic review and meta-analysis. *JAMA Netw Open*. 2021;4(8):e2120736

10. Martí-Carreras J, Maes P. Human cytomegalovirus genomics and transcriptomics through the lens of next-generation sequencing: revision and future challenges. *Virus Genes*. 2019;55(2):138–164

11. Cudini J, Roy S, Houldcroft CJ, et al. Human cytomegalovirus haplotype reconstruction reveals high diversity due to superinfection and evidence of within-host recombination. *Proc Natl Acad Sci USA*. 2019;116(12):5693–5698

12. Ross SA, Arora N, Novak Z, Fowler KB, Britt WJ, Boppana SB. Cytomegalovirus reinfections in healthy seroimmune women. *J Infect Dis*. 2010;201(3):386–389

13. Renzette N, Gibson L, Bhattacharjee B, et al. Rapid intrahost evolution of human cytomegalovirus is shaped by demography and positive selection. *PLoS Genet*. 2013;9(9):e1003735

14. Patro ARK. Subversion of immune response by human cytomegalovirus. *Front Immunol*. 2019;10:1155

15. Cheng S, Caviness K, Buehler J, Smithey M, Nikolich-Žugich J, Goodrum F. Transcriptome-wide characterization of human cytomegalovirus in natural infection and experimental latency. *Proc Natl Acad Sci USA*. 2017;114(49):E10586–E10595

16. Tirosh O, Cohen Y, Shitrit A, et al. The transcription and translation landscapes during human cytomegalovirus infection reveal novel host-pathogen interactions. *PLoS Pathog*. 2015;11(11):e1005288

17. Ouellette CP, Sánchez PJ, Xu Z, et al. Blood genome expression profiles in infants with congenital cytomegalovirus infection. *Nat Commun*. 2020;11(1):3548

18. Arav-Boger R. Strain variation and disease severity in congenital cytomegalovirus infection: in search of a viral marker. *Infect Dis Clin North Am*. 2015;29(3):401–414

19. Dobbins GC, Patki A, Chen D, et al. Association of CMV genomic mutations with symptomatic infection and hearing loss in congenital CMV infection. *BMC Infect Dis*. 2019;19(1):1046

20. Forte E, Zhang Z, Thorp EB, Hummel M. Cytomegalovirus latency and reactivation: an intricate interplay with the host immune response. *Front Cell Infect Microbiol*. 2020;10:130

21. Griffiths P, Reeves M. Pathogenesis of human cytomegalovirus in the immunocompromised host. *Nat Rev Microbiol*. 2021;19(12):759–773

22. Permar SR, Schleiss MR, Plotkin SA. Advancing our understanding of protective maternal immunity as a guide for development of vaccines to reduce congenital cytomegalovirus infections. *J Virol*. 2018;92(7):e00030–e00018

23. Leruez-Ville M, Foulon I, Pass R, Ville Y. Cytomegalovirus infection during pregnancy: state of the science. *Am J Obstet Gynecol*. 2020;223(3):330–349

24. Pesch MH, Kuboushek K, McKee MM, Thorne MC, Weinberg JB. Congenital cytomegalovirus infection. *BMJ*. 2021;373:n1212

25. Boppana SB, Pass RF, Britt WJ, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. *Pediatr Infect Dis J*. 1992;11(2):93–99

26. Bartlett AW, McMullan B, Rawlinson WD, Palasanthiran P. Hearing and neurodevelopmental outcomes for children with asymptomatic congenital cytomegalovirus infection: a systematic review [published online ahead of print September 6, 2017]. *Rev Med Virol*. doi:10.1002/rmv.1938

27. Rawlinson WD, Boppana SB, Fowler KB, et al. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. *Lancet Infect Dis*. 2017;17(6):e177–e188

28. Ronchi A, Zeray F, Lee LE, et al. Evaluation of clinically asymptomatic high risk infants with congenital cytomegalovirus infection. *J Perinatol*. 2020;40(1):89–96

29. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev Med Virol*. 2007;17(5):355–363

30. Bristow BN, O'Keefe KA, Shafir SC, Sorvillo FJ. Congenital cytomegalovirus mortality in the United States, 1990–2006. *PLoS Negl Trop Dis*. 2011;5(4):e1140

31. Fletcher KT, Horrell EMW, Ayugi J, et al. The natural history and rehabilitative outcomes of hearing loss in congenital cytomegalovirus: a systematic review. *Otol Neurotol*. 2018;39(7):854–864

32. Fowler KB, Ross SA, Shimamura M, et al. Racial and ethnic differences in the prevalence of congenital cytomegalovirus infection. *J Pediatr*. 2018;200:196-201.e1

33. Inagaki K, Blackshear C, Palmer A, Hobbs CV. Risk factors, geographic distribution, and healthcare burden of symptomatic congenital cytomegalovirus infection in the United States: analysis of a nationally representative database, 2000-2012. *J Pediatr*. 2018;199:118-123.e1

34. Lantos PM, Hoffman K, Permar SR, Jackson P, Hughes BL, Swamy GK. Geographic disparities in cytomegalovirus infection during pregnancy. *J Pediatric Infect Dis Soc.* 2017;6(3):e55–e61

35. Page JM, Bardsley T, Thorsten V, et al. Stillbirth associated with infection in a diverse US cohort. *Obstet Gynecol.* 2019;134(6):1187–1196

36. Iwasenko JM, Howard J, Arbuckle S, et al. Human cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy. *J Infect Dis.* 2011;203(11): 1526–1533

37. Shi TL, Huang L-J, Xiong Y-Q, et al. The risk of herpes simplex virus and human cytomegalovirus infection during pregnancy upon adverse pregnancy outcomes: a meta-analysis. *J Clin Virol.* 2018;104:48–55

38. Leruez-Ville M, Magny J-F, Couderc S, et al. Risk factors for congenital cytomegalovirus infection following primary and nonprimary maternal infection: a prospective neonatal screening study using polymerase chain reaction in saliva. *Clin Infect Dis.* 2017;65(3):398–404

39. Grosjean J, Trapes L, Hantz S, et al. Human cytomegalovirus quantification in toddlers saliva from day care centers and emergency unit: a feasibility study. *J Clin Virol.* 2014;61(3):371–377

40. Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. *Rev Med Virol.* 2011;21(4):240–255

41. Hughes BL, Gans KM, Raker C, Hipolito ER, Rouse DJ. A brief prenatal intervention of behavioral change to reduce the risk of maternal cytomegalovirus: a randomized controlled trial. *Obstet Gynecol.* 2017;130(4):726–734

42. Schaefer MR, Holttum J, Olson M, et al. Development and assessment of a prenatal cytomegalovirus (CMV) educational survey: implementation and impact in a metropolitan university-based clinic. *Int J Womens Health.* 2020;12:1205–1214

43. Cannon MJ, Levis DM, McBride H, et al. Family perceptions of newborn cytomegalovirus screening: a qualitative study. *Int J Neonatal Screen.* 2021;7(4):80

44. Jeon J, Victor M, Adler SP, et al. Knowledge and awareness of congenital cytomegalovirus among women. *Infect Dis Obstet Gynecol.* 2006:80383

45. Barton M, Forrester AM, McDonald J. Update on congenital cytomegalovirus infection: prenatal prevention, newborn diagnosis, and management. *Paediatr Child Health.* 2020;25(6):395–396

46. Johnson J, Anderson B, Pass RF. Prevention of congenital cytomegalovius infection. *Clin Obstet Gynecol.* 2012;55(2): 521-530

47. Barber V, Calvert A, Vandrevala T, et al. Prevention of acquisition of cytomegalovirus infection in pregnancy through hygiene-based behavioural interventions: a systematic review and gap analysis. *Pediatr Infect Dis J.* 2020;39(10):949–954

48. Luck SE, Wieringa JW, Blázquez-Gamero D, et al; ESPID Congenital CMV Group Meeting, Leipzig 2015. Congenital cytomegalovirus: a European expert consensus statement on diagnosis and management. *Pediatr Infect Dis J.* 2017;36(12):1205–1213

49. Fowler K, Eunice Kennedy Shriver National Institute of Child Health and Human Development. Prenatal behavioral intervention to prevent maternal cytomegalovirus (CMV) in pregnancy. Available at: https://ClinicalTrials.gov/show/ NCT04615715. Accessed January 5, 2022

50. Fernandez C, Chasqueira M-J, Marques A, et al. Lower prevalence of congenital cytomegalovirus infection in Portugal: possible impact of COVID-19 lockdown? *Eur J Pediatr.* 2021;181(3):1–4

51. Hutton J, Rowan PJ. Vertical transmission and discordance of cytomegalovirus in twin pregnancies. *Front Cell Infect Microbiol.* 2021;11:676988

52. Wujcicka W, Paradowska E, Studzińska M, Wilczyński J, Nowakowska D. TLR2 2258 G:a single nucleotide polymorphism and the risk of congenital infection with human cytomegalovirus. *Virol J.* 2017;14(1):12

53. Wujcicka W, Paradowska E, Studzińska M, Wilczyński J, Nowakowska D. Toll-like receptors genes polymorphisms and the occurrence of HCMV infection among pregnant women. *Virol J.* 2017;14(1):64

54. Devlieger R, Buxmann H, Nigro G, et al. Serial monitoring and hyperimmunoglobulin versus standard of care to prevent congenital cytomegalovirus infection: a phase III randomized trial. *Fetal Diagn Ther.* 2021;48(8):611–623

55. Hughes BL, Clifton RG, Rouse DJ, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network. A trial of hyperimmune globulin to prevent congenital cytomegalovirus infection. *N Engl J Med.* 2021;385(5):436–444

56. Minsart A-F, Smiljkovic M, Renaud C, et al. Use of cytomegalovirus-specific hyperimmunoglobulins in pregnancy: a retrospective cohort. *J Obstet Gynaecol Can.* 2018;40(11):1409–1416

57. Shahar-Nissan K, Pardo J, Peled O, et al. Valaciclovir to prevent vertical transmission of cytomegalovirus after maternal primary infection during pregnancy: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2020;396(10253): 779–785

58. Leruez-Ville M, Ghout I, Bussières L, et al. In utero treatment of congenital cytomegalovirus infection with valacyclovir in a multicenter, open-label, phase II study. *Am J Obstet Gynecol.* 2016;215(4): 462.e461-462.e410

59. Fowler KB, McCollister FP, Sabo DL, et al; CHIMES Study. A targeted approach for congenital cytomegalovirus screening within newborn hearing screening. *Pediatrics.* 2017;139(2):e20162128

60. National CMV. Advocacy in action. Available at: www.nationalcmv.org/about-us/ advocacy. Accessed on October 22, 2021

61. Grosse SD, Dollard SC, Ortega-Sanchez IR. Economic assessments of the burden of congenital cytomegalovirus infection and the cost-effectiveness of prevention strategies. Paper presented at: Seminars in Perinatology2021.

62. Tastad KJ, Schleiss MR, Lammert SM, Basta NE. Awareness of congenital cytomegalovirus and acceptance of maternal and newborn screening. *PLoS One.* 2019;14(8):e0221725

63. Din ES, Brown CJ, Grosse SD, et al. Attitudes toward newborn screening for cytomegalovirus infection. *Pediatrics.* 2011;128(6):e1434–e1442

64. Gantt S, Goldfarb DM, Park A, et al. Performance of the Alethia CMV assay for detection of cytomegalovirus by use of neonatal saliva swabs. *J Clin Microbiol.* 2020;58(4):e01951–e01919

65. Ross SA, Michaels MG, Ahmed A, et al. Contribution of breastfeeding to false-positive saliva polymerase chain reaction for newborn congenital cytomegalovirus screening. *J Infect Dis.* 2018; 217(10):1612–1615

66. Dollard SC, Dreon M, Hernandez-Alvarado N, et al. Sensitivity of dried blood spot testing for detection of congenital cytomegalovirus infection. *JAMA Pediatr.* 2021;175(3):e205441

67. Pesch MH, Saunders NA, Abdelnabi S. Cytomegalovirus infection in pregnancy: prevention, presentation, management and neonatal outcomes. *J Midwifery Womens Health.* 2021;66(3):397–402

68. Joint Committee on Infant Hearing. Year 2019 position statement: principles and guidelines for early hearing detection and intervention programs. *J Early Hear Detect Interv.* 2019;4(2):1–44

69. Demmler-Harrison GJ, Miller JA, Group HCCLS; Houston Congenital Cytomegalovirus Longitudinal Study Group. Maternal cytomegalovirus immune status and hearing loss outcomes in congenital cytomegalovirus-infected offspring. *PLoS One.* 2020;15(10):e0240172

70. Foulon I, De Brucker Y, Buyl R, et al. Hearing loss with congenital cytomegalovirus infection. *Pediatrics.* 2019;144(2): e20183095

71. Dahle AJ, Fowler KB, Wright JD, Boppana SB, Britt WJ, Pass RF. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. *J Am Acad Audiol.* 2000;11(5):283–290

72. Lanzieri TM, Chung W, Flores M, et al; Congenital Cytomegalovirus Longitudinal Study Group. Hearing loss in children with asymptomatic congenital cytomegalovirus infection. *Pediatrics.* 2017;139(3): e20162610

73. Jin HD, Demmler-Harrison GJ, Coats DK, et al; Congenital CMV Longitudinal Study Group. Long-term visual and ocular sequelae in patients with congenital cytomegalovirus infection. *Pediatr Infect Dis J.* 2017;36(9):877–882

74. Capretti MG, Marsico C, Guidelli Guidi S, et al. Neonatal and long-term ophthalmological findings in infants with symptomatic and asymptomatic congenital cytomegalovirus infection. *J Clin Virol.* 2017;97:59–63

75. Coats DK, Demmler GJ, Paysse EA, Du LT, Libby C, Group CCLS. Ophthalmologic findings in children with congenital cytomegalovirus infection. *J AAPOS.* 2000; 4(2):110–116

76. Kwak M, Yum MS, Yeh HR, Kim HJ, Ko TS. Brain magnetic resonance imaging findings of congenital cytomegalovirus infection as a prognostic factor for neurological outcome. *Pediatr Neurol.* 2018;83:14–18

77. Lucignani G, Rossi Espagnet MC, Napolitano A, et al. A new MRI severity score to predict long-term adverse neurologic outcomes in children with congenital cytomegalovirus infection. *J Matern Fetal Neonatal Med.* 2019;34(6):859–866

78. Lyutenski S, Götz F, Giourgas A, et al. Does severity of cerebral MRI lesions in congenital CMV infection correlates with the outcome of cochlear implantation? *Eur Arch Otorhinolaryngol.* 2017;274(3): 1397–1403

79. Castellanos ME, de la Mata Navazo S, Bermejo MC, et al. Association between neuroimaging findings and neurological sequelae in patients with congenital cytomegalovirus infection [published online ahead of print March 8, 2019]. *Neurología (Engl Ed).* doi: 10.1016/ j.nrl.2018.11.003

80. Oosterom N, Nijman J, Gunkel J, et al. Neuro-imaging findings in infants with congenital cytomegalovirus infection: relation to trimester of infection. *Neonatology.* 2015;107(4):289–296

81. Noyola DE, Demmler GJ, Nelson CT, et al; Houston Congenital CMV Longitudinal Study Group. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. *J Pediatr.* 2001;138(3):325–331

82. Lucignani G, Rossi Espagnet MC, Napolitano A, et al. A new MRI severity score to predict long-term adverse neurologic outcomes in children with congenital cytomegalovirus infection. *J Matern Fetal Neonatal Med.* 2021;34(6):859–866

83. Blázquez-Gamero D, Soriano-Ramos M, Martínez de Aragón A, et al; Spanish Registry of Infants with Congenital Cytomegalovirus Infection (REDICCMV) Study Group. Role of magnetic resonance imaging and cranial ultrasonography in congenital cytomegalovirus infection. *Pediatr Infect Dis J.* 2019;38(11):1131–1137

84. Keymeulen A, De Leenheer E, Casaer A, et al. Cranial ultrasound and MRI: complementary or not in the diagnostic assessment of children with congenital CMV infection? *Eur J Pediatr.* 2021; 181(3):911–920

85. Vande Walle C, Keymeulen A, Schiettecatte E, et al. Brain MRI findings in newborns with congenital cytomegalovirus infection: results from a large cohort study. *Eur Radiol.* 2021;31(10):8001–8010

86. Roee B, Adi W, Michael B, et al. Subtle findings on fetal brain imaging in CMV infected pregnancies: what is the clinical significance? a retrospective analysis with outcome correlation. *Prenat Diagn.* 2020;40(4):447–453

87. Gaur P, Kachramanoglou C, Lyall H, Jan W. Is it not time for international guidelines to combat congenital cytomegalovirus infection? a review of central nervous system manifestations. *Clin Radiol.* 2020;75(8):644.e647-644.e616

88. Brown RM, Rana PSJB, Jaeger HK, O'Dowd JM, Balemba OB, Fortunato EA. Human cytomegalovirus compromises development of cerebral organoids. *J Virol.* 2019;93(17):e00957–e00919

89. Chatzakis C, Ville Y, Makrydimas G, Dinas K, Zavlanos A, Sotiriadis A. Timing of primary maternal cytomegalovirus infection and rates of vertical transmission and fetal consequences. *Am J Obstet Gynecol.* 2020;223(6):870–883.e11

90. Faure-Bardon V, Magny JF, Parodi M, et al. Sequelae of congenital cytomegalovirus following maternal primary infections are limited to those acquired in the first trimester of pregnancy. *Clin Infect Dis.* 2019;69(9):1526–1532

91. Lanari M, Capretti MG, Lazzarotto T, et al. Neuroimaging in CMV congenital infected neonates: how and when. *Early Hum Dev.* 2012;88(Suppl 2):S3–S5

92. Rolland M, Martin H, Bergamelli M, et al. Human cytomegalovirus infection is associated with increased expression of the lissencephaly gene PAFAH1B1 encoding LIS1 in neural stem cells and congenitally infected brains. *J Pathol.* 2021;254(1):92–102

93. Kuan MI, Jaeger HK, Balemba OB, et al. Human cytomegalovirus interactions with the basement membrane protein nidogen 1. *J Virol.* 2021;95(3):e01506-e01520

94. Krstanović F, Britt WJ, Jonjić S, Brizić I. Cytomegalovirus infection and inflammation in developing brain. *Viruses*. 2021; 13(6):1078

95. Moulden J, Sung CYW, Brizic I, Jonjic S, Britt W. Murine models of central nervous system disease following congenital human cytomegalovirus infections. *Pathogens*. 2021;10(8):1062

96. Teissier N, Fallet-Bianco C, Delezoide A-L, et al. Cytomegalovirus-induced brain malformations in fetuses. *J Neuropathol Exp Neurol*. 2014;73(2):143–158

97. Gabrielli L, Bonasoni MP, Santini D, et al. Human fetal inner ear involvement in congenital cytomegalovirus infection. *Acta Neuropathol Commun*. 2013;1:63

98. Carraro M, Almishaal A, Hillas E, Firpo M, Park A, Harrison RV. Cytomegalovirus (CMV) infection causes degeneration of cochlear vasculature and hearing loss in a mouse model. *J Assoc Res Otolaryngol*. 2017;18(2):263–273

99. Kimberlin DW, Jester PM, Sánchez PJ, et al; National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. Valganciclovir for symptomatic congenital cytomegalovirus disease. *N Engl J Med*. 2015;372(10): 933–943

100. Ziv L, Yacobovich J, Pardo J, et al. Hematologic adverse events associated with prolonged valganciclovir treatment in congenital cytomegalovirus infection. *Pediatr Infect Dis J*. 2019;38(2):127–130

101. Food and Drug Administration. FDA-approved cochlear implants. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfRL/rl.cfm. Accessed December 23, 2021

102. Yoshinaga-Itano C, Sedey AL, Wiggin M, Mason CA. Language outcomes improved through early hearing detection and earlier cochlear implantation. *Otol Neurotol*. 2018;39(10):1256–1263

103. Kraaijenga VJC, Van Houwelingen F, Van der Horst SF, et al. Cochlear implant performance in children deafened by congenital cytomegalovirus-a systematic review. *Clin Otolaryngol*. 2018;43(5): 1283–1295

104. Lopez AS, Lanzieri TM, Claussen AH, et al; Congenital Cytomegalovirus Longitudinal Study Group. Intelligence and academic achievement with asymptomatic congenital cytomegalovirus infection. *Pediatrics*. 2017;140(5):e20171517

105. Pinninti S, Christy J, Almutairi A, Cochrane G, Fowler KB, Boppana S. Vestibular, gaze, and balance disorders in asymptomatic congenital cytomegalovirus infection. *Pediatrics*. 2021;147(2): e20193945

106. Lanzieri TM, Leung J, Caviness AC, et al. Long-term outcomes of children with symptomatic congenital cytomegalovirus disease. *J Perinatol*. 2017;37(7):875–880

107. Smithers-Sheedy H, Raynes-Greenow C, Badawi N, et al. Congenital cytomegalovirus among children with cerebral palsy. *J Pediatr*. 2017;181:267-271.e21

108. Gentile I, Zappulo E, Riccio MP, et al. Prevalence of congenital cytomegalovirus infection assessed through viral genome detection in dried blood spots in children with autism spectrum disorders. *In Vivo*. 2017;31(3):467–473

109. Zhou W, Wang W. Fast-spreading SARS-CoV-2 variants: challenges to and new design strategies of COVID-19 vaccines. *Signal Transduct Target Ther*. 2021;6(1):226

110. Chen DH, Jiang H, Lee M, Liu F, Zhou ZH. Three-dimensional visualization of tegument/capsid interactions in the intact human cytomegalovirus. *Virology*. 1999;260(1):10–16

111. Gibson L, Piccinini G, Lilleri D, et al. Human cytomegalovirus proteins pp65 and immediate early protein 1 are common targets for CD8+ T cell responses in children with congenital or postnatal human cytomegalovirus infection. *J Immunol*. 2004;172(4):2256–2264

112. Anderholm KM, Bierle CJ, Schleiss MR. Cytomegalovirus vaccines: current status and future prospects. *Drugs*. 2016;76(17):1625–1645

113. Cheeran MC-J, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev*. 2009;22(1):99–126

114. Elkan Miller T, Weisz B, Yinon Y, et al. Congenital cytomegalovirus infection following second and third trimester maternal infection is associated with mild childhood adverse outcome not predicted by prenatal imaging. *J Pediatric Infect Dis Soc*. 2021;10(5): 562–568

115. National Institute of Allergy and Infectious Diseases. Asymptomatic Congenital CMV Treatment. ClinicalTrials.gov Identifier: NCT03301415. Available at: https://clinicaltrials.gov/ct2/show/NCT03301415. Accessed December 21, 2021

116. Park A, National Institute on Deafness and Other Communication Disorders, Genentech Inc. Randomized controlled trial of valganciclovir for cytomegalovirus infected hearing impaired infants (ValEAR). ClinicalTrials.gov Identifier: NCT03107871. Available at: https://clinicaltrials.gov/ct2/show/NCT03107871. Accessed December 21, 2021

117. ACTM V. Congenital cytomegalovirus: efficacy of antiviral treatment (CONCERT 2). ClinicalTrials.gov identifier: NCT02005822. Available at: https://clinicaltrials.gov/ct2/show/NCT02005822. Accessed December 21, 2021

118. Ville Y. Assistance publique - Hôpitaux de Paris F. prenatal treatment of congenital cytomegalovirus infection with letermovir randomized against valaciclovir - STEP 1 (CYMEVAL3-STEP1). ClinicalTrials.gov identifier: NCT04732260. Available at: https://ClinicalTrials.gov/show/NCT04732260. Accessed December 21, 2021

119. Merck Sharp & Dohme Corp. V160 2-dose and 3-dose regimens in healthy cytomegalovirus (CMV) seronegative females (V160-002). ClinicalTrials.gov identifier: NCT03486834. Available at: https://ClinicalTrials.gov/show/NCT03486834. Accessed December 21, 2021

120. GlaxoSmithKline. A study of safety and immune response to different doses of a cytomegalovirus vaccine in healthy adults. ClinicalTrials.gov identifier NCT05089630. Available at: https://ClinicalTrials.gov/show/NCT05089630. Accessed December 21, 2021

121. Moderna Tx Inc. A study to evaluate the efficacy, safety, and immunogenicity of mRNA-1647 cytomegalovirus (CMV) vaccine in healthy participants 16 to 40 years of age. ClinicalTrials.gov identifier: NCT05085366. Available at: https://ClinicalTrials.gov/show/NCT05085366. Accessed December 21, 2021