



Progress toward Development of a Vaccine against Congenital Cytomegalovirus Infection

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ABSTRACT A vaccine against congenital human cytomegalovirus (CMV) infection is a major public health priority. Congenital CMV causes substantial long-term morbidity, particularly sensorineural hearing loss (SNHL), in newborns, and the public health impact of this infection on maternal and child health is underrecognized. Although progress toward development of a vaccine has been limited by an incomplete understanding of the correlates of protective immunity for the fetus, knowledge about some of the key components of the maternal immune response necessary for preventing transplacental transmission is accumulating. Moreover, although there have been concerns raised about observations indicating that maternal seropositivity does not fully prevent recurrent maternal CMV infections during pregnancy, it is becoming increasingly clear that preconception immunity does confer some measure of protection against both CMV transmission and CMV disease (if transmission occurs) in the newborn infant. Although the immunity to CMV conferred by both infection and vaccination is imperfect, there are encouraging data emerging from clinical trials demonstrating the immunogenicity and potential efficacy of candidate CMV vaccines. In the face of the knowledge that between 20,000 and 30,000 infants are born with congenital CMV in the United States every year, there is an urgent and compelling need to accelerate the pace of vaccine trials. In this minireview, we summarize the status of CMV vaccines in clinical trials and provide a perspective on what would be required for a CMV immunization program to become incorporated into clinical practice.

KEYWORDS congenital infections, cytomegalovirus, placental immunology, vaccines

Congenital infection with human cytomegalovirus (CMV) infection is common. An estimate of its prevalence gleaned from a meta-analysis indicated that approximately 0.65% of all newborns in the United States have congenital CMV infection (1). A more recent, prospective multicenter study identified a birth prevalence of congenital CMV of ~0.5% (2). Although the majority of these infections are asymptomatic, at least 10% of the 20,000 to 30,000 CMV-infected infants born in the United States annually have long-term neurodevelopmental sequelae, including mental retardation, seizure disorders, cerebral palsy, sensorineural hearing loss (SNHL), microcephaly, and learning disabilities (3). Clinical manifestations in symptomatic infants include growth retardation, petechiae, hepatosplenomegaly, microcephaly, jaundice, seizures, rash, and periventricular calcifications (4). Infants that are symptomatic at birth are at greater risk for neurodevelopmental sequelae. Of these sequelae, SNHL is the most common. Between 22% and 65% of children with symptomatic disease at birth, and 6% to 23% of children with asymptomatic congenital CMV infection, have or eventually develop SNHL following congenital CMV infection (5–7). CMV-induced SNHL may be

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present at birth or may become clinically evident later in childhood (8, 9). Overall, it is estimated that congenital CMV infection is responsible for over 20% of all pediatric SNHL observed at birth (10). Hearing deficits secondary to CMV are more common than those caused by congenital infection with rubella, or by meningitis due to *Haemophilus influenzae* type B, in their historical respective prevaccine peak years (11). Given the magnitude of the impact of congenital CMV, and the lifelong nature of disabilities associated with this infection, the economic impact on society is substantial (12–14).

In recent years there has been increased emphasis on the potential economic benefits of a vaccine against congenital CMV. The National Academy of Medicine (NAM), in a report published in 2000 (14), identified the discovery of a hypothetical CMV vaccine that would be administered to 12-year-olds for the prevention of congenital infection as a “level 1” (most favorable) priority. Using quality-adjusted life-years as the metric for analysis, the NAM task force concluded that the introduction of an efficacious CMV vaccine capable of preventing congenital infection—and therefore the lifelong disability associated with congenital CMV—would be highly cost-effective. It has now been over 15 years since the publication of this report, but no CMV vaccine has yet been licensed. This minireview gauges the progress that has been made toward the goal of development of a CMV vaccine against congenital infection, and highlights recent and current clinical trials of vaccine candidates. Barriers to licensure of a CMV vaccine are identified, and recommendations are provided for high-priority areas of research that are required to address this unsolved public health problem.

CORRELATES OF PROTECTIVE MATERNAL IMMUNITY AND POTENTIAL FOR VACCINES

Ideally, development of an effective congenital CMV vaccine would be informed by knowledge about key correlates of protective immunity required to block transmission of the virus to the fetus. Fortunately, a number of aspects of the maternal immune response have been identified that play a role in both preventing congenital CMV infection and ameliorating the severity of CMV disease if vertical transmission occurs (15, 16). Although the necessary and sufficient correlates of the protective maternal immune response to CMV require better elucidation, there is clear evidence that maternal antibody and T cell responses are associated with protection against transmission (17–21). This knowledge is balanced against the emerging recognition that preconception maternal seropositivity to CMV is insufficient to provide complete protection against recurrent infections that can also, like primary infections, result in congenital transmission during pregnancy. While congenital transmission in mothers with preexisting immunity occurs at a low rate, because of the high rates of maternal seropositivity (particularly in low- and middle-income countries), transmission to the fetuses of seropositive mothers is globally the most common form of congenital CMV infection. Indeed, most congenital infections occur in the context of nonprimary (recurrent) maternal infection worldwide (22–25). It has been estimated that approximately 75% of congenital CMV infections occur in the setting of recurrent maternal infection during pregnancy (24). Maternal recurrent infections may be associated with reactivation of latent virus but have also been suggested to be due to exogenous reinfections with new strains of CMV. Some of these reinfections may occur between pregnancies. Evidence for the reinfection mechanism comes from studies demonstrating the development of new antibody specificities with respect to virally encoded envelope glycoproteins in sequential pregnancies and, in some instances, from molecular data confirming the acquisition of a new strain of virus (26). This knowledge complicates vaccine design, but should not negatively affect the progress that has been made in defining correlates of protective immunity, as reviewed below.

Although there is increasing evidence for recurrent maternal infection as a major mechanism of congenital CMV infection, an issue of critical importance is whether the risk of neurodevelopmental sequelae is reduced in the context of congenital transmission that occurs in the setting of preexisting (preconception) maternal immunity in women with recurrent infection. This question is, of course, of paramount importance

with respect to the issue of vaccination, since a maternal vaccine that reduces the magnitude of CMV disease in an infant would be judged a success, even if occasional transmission occurred. Some experts have expressed the view that there is no evidence that maternal immunity to CMV provides protection against either congenital infection or the long-term sequelae associated with congenital transmission (27). However, it is clear that the risks of transmission in the context of primary infection are strikingly different from those seen in the context of recurrent infection. Primary infections result in CMV transmission in approximately 30% of affected pregnancies (28, 29), and prospective studies have demonstrated that preexisting maternal immunity confers a 69% reduction of the risk of congenital CMV in future pregnancies (30). Thus, although prior maternal infection with CMV does not provide complete protection against transmission to the fetus, it does clearly reduce the risk. Indeed, two recent prospective studies both indicated a protective effect of maternal immunity against congenital CMV infection, with highly significantly reduced rates of vertical transmission in women with nonprimary compared to primary infections (31, 32). Thus, although imperfect, there is evidence to support the contention that preconception maternal immunity to CMV is a barrier to vertical transmission. Moreover, there is evidence demonstrating that sequelae are reduced, even if transmission occurs, in the setting of preconception maternal immunity. This has been demonstrated in particular for SNHL, where both the severity and risk of progression of hearing loss are more substantial in infected infants born to transmitting mothers with primary CMV infections during pregnancy than in those infants acquiring congenital CMV in the context of recurrent maternal infection (33).

Although more research is needed on the impact of preconception maternal immunity on congenital CMV transmission and on the relative risks of primary and nonprimary maternal infections with respect to fetal transmission, there is emerging information about specific correlates of protective immunity that helps to inform the finer details of CMV vaccine design, both from animal models as well as descriptive studies in women. Virus-neutralizing antibodies targeting the immunodominant CMV glycoprotein B (gB), as well as the glycoprotein H/L (gH/gL) complex, have been shown to prevent CMV transmission in a guinea pig CMV (GPCMV) congenital infection model (34–37). Moreover, passively administered polyclonal anti-CMV IgG, even in the absence of T cell responses, demonstrated protective capacity in a rhesus macaque CMV (RhCMV) model of congenital infection (38). In addition, antibodies against another CMV glycoprotein complex involved in epithelial and endothelial cell entry of virus—the “pentameric complex” (PC) of glycoproteins gH/gL/UL128/130/131—have been shown to cross-neutralize diverse clinical isolates of CMV (39), an important observation in light of the role that recurrent infection (due, at least in some cases, to reinfection with a new strain in a previously “immune” woman) plays in congenital CMV transmission. Antibodies against the PC, as well as the magnitude of the CMV IgG avidity index (AI), have both been identified as potential correlates of protection against congenital CMV transmission (40), providing quantifiable surrogates for protection that can be prospectively monitored in clinical vaccine trials.

In addition to antibody responses, the CD4⁺ T cell response has been demonstrated, via depletion studies in the RhCMV model, to be an important contributor to protection against placental virus transmission and fetal disease (41, 42). The relevance of these rhesus macaque studies to humans was underscored by the recent report of the evolution of CMV-specific T cell immunity in women with documented primary CMV infection during pregnancy (43). In this study, 15 (34%) of 44 women with a documented primary CMV infection gave birth to infants with congenital CMV infection, but when immune responses of transmitting and nontransmitting women were compared, the magnitude of the CD4⁺ T cell response to the CMV tegument phosphoprotein pp65 (ppUL83) was significantly higher in nontransmitting mothers than in transmitting mothers. Those investigators also observed a higher IgG avidity index (AI) in nontransmitting mothers (43). That study strongly suggested—for the first time—a clear role for pp65-specific CD4⁺ T cell responses in prevention of congenital CMV transmission.

These results have important implications for subunit vaccine design for future clinical trials. Notably, many candidate subunit and vectored vaccine platforms (described in greater detail below) include pp65.

In addition to the importance of the maternal immune response, there are interesting data emerging that demonstrate that the size of the dose of CMV to which women are exposed may have an impact on the likelihood of subsequent congenital transmission. This may be particularly relevant to the issue of the risk of maternal reinfection during pregnancy. Challenge studies have shown that large doses of CMV can overcome prior immunity that would otherwise protect against lower doses of virus (44). Moreover, the rapidity with which containment of CMV replication and systemic viremia occurs after primary infection may play an important role in predicting placental transmission (38). Although CMV is described as a slowly replicating virus in cell culture, analysis of the dynamics of replication *in vivo* suggests that the doubling time of the virus is actually much shorter, on the order of 1 day in immunocompromised patients (45). Similar analyses of the impact of vaccines on replication dynamics in pregnant women are needed and could be useful in providing insights into mechanisms of protection against placental and congenital transmission.

Recently completed or currently active clinical trials of candidate CMV vaccines are summarized in Table 1. Vaccines in preclinical development that target human CMV but that have not yet been evaluated in volunteers are also separately considered in Table 1. General categories of CMV vaccines included adjuvanted recombinant protein vaccines targeting gB; vaccines targeting gB alone or in combination with UL83 (pp65) and (in some cases) the major immediate early protein 1 (IE1), generated using viral vectors or based on generation of virus-like particles (VLPs); replication-impaired or replication-defective CMV vaccines (live, attenuated, or disabled infectious single-cycle [DISC] vaccines); and other novel platforms, such as dense body (DB) vaccines. These individual categories, as well as the current stage of development of these vaccines (clinical trials or preclinical studies), are summarized below.

PURIFIED RECOMBINANT gB SUBUNIT VACCINES

Subunit approaches utilizing adjuvanted recombinant formulations of gB have arguably advanced the furthest in clinical trials of CMV vaccines to date. Several phase I and phase II clinical trials utilizing a recombinant CMV gB in microfluidized adjuvant 59 (MF59), a proprietary oil-in-water emulsion from Novartis (first used in influenza vaccines), have been completed (46–50). Most of the studies have utilized a three-dose series of vaccine. The gB vaccine adjuvanted with MF59 is expressed as a truncated, secreted polypeptide, and the protein is purified by chromatography from tissue culture supernatants in Chinese hamster ovary (CHO) cells. It is unclear whether the conformation of this truncated, secreted, and uncleaved form of gB recapitulates the conformation of gB antigen expressed on virions and/or the surface of CMV-infected cells. In this regard, other forms of gB (discussed below) may retain a more authentic conformation which, in turn, may allow expression of conformational epitopes important in protective vaccine responses. The conformation of gB that maximizes vaccine responsiveness and protection is an important area for future study.

The gB/MF59 vaccine has demonstrated encouraging results in clinical trials. In a phase II study in postpartum women, the gB/MF59 vaccine demonstrated 50% efficacy against primary CMV infection in seronegative women vaccinated within 1 year of giving birth compared to women in the same cohort who received the placebo (47). This was indeed a landmark study, insofar as it was the first trial demonstrating the efficacy of any vaccine for preventing primary CMV infection, an important milestone in progress toward maternal immunization against congenital CMV transmission. Women who enrolled in this study but were found to already be ELISA antibody-positive for CMV were also vaccinated with either the gB/MF59 vaccine or the placebo; gB-specific responses were shown to be boosted in vaccinated seropositive women compared to controls, even in the face of this preexisting immunity, a finding

TABLE 1 Human CMV vaccine candidates in various stages of preclinical and clinical development^a

Category	Characteristics
Vaccines previously evaluated in clinical trials: not currently undergoing development	
AD169 vaccine	Engendered CMV-specific antibodies in seronegative vaccinees Injection site and systemic reactogenicity
Towne vaccine (\pm rhIL-12)	Elicitation of humoral and cellular immune responses Safe; no latent infection or viral shedding in recipients Reduction in CMV disease in renal transplant recipients Coadministrated with recombinant IL-12 in phase I studies
Towne/Toledo chimera vaccines	No evidence for latency or shedding in recipients in phase I studies Attenuated compared to Toledo strain of CMV Variable immunogenicity in seronegatives A mutation in <i>UL128</i> abrogates formation of the PC in all four chimeras
Subunit recombinant glycoprotein B (gB) (CHO cell expression), MF59/AS01 adjuvant	Favorable safety evaluation in phase I Neutralizing antibody and cell-mediated immune responses (limited to CD4 ⁺) Boost of humoral immunity in seropositive recipients (gB/MF59) Demonstrated efficacy in phase II studies in young women with respect to protection against primary CMV infection, and against development of CMV disease in seronegative SOT recipients with seropositive donors (gB/MF59)
Alphavirus-vectored gB/pp65/IE1 vaccine	Venezuelan equine encephalitis (VEE) vector Towne gB (extracellular domain) and pp65-IE1 fusion protein expressed in a double-promoter replicon construct Replication-deficient, virus-like replicon particles (VRP)
Canarypox-vectored glycoprotein B vaccine	Humoral and cellular immune responses Favorable safety profile No augmentation of immunogenicity in "prime-boost" study with gB/MF59 "Prime-boost" effect when administered with Towne vaccine
Canarypox-vectored pp65 (UL83) vaccine	Favorable safety profile Strong antibody and CD8 ⁺ cell-mediated immune responses (phase I)
CMV vaccines currently undergoing evaluation in clinical trials	
V160-001 replication-defective vaccine	AD169 backbone with restoration UL129/130/131 PC components Disabled, single-cycle vaccine rendered replication incompetent by inclusion of ddFKBP/Shld1 in vaccine design Administered with alum-based adjuvant Phase I studies fully enrolled and data currently under analysis
PADRE-pp65-CMV fusion peptide vaccines \pm CpG DNA adjuvant	Lipidated fusion peptides constructed from pp65 CTL epitopes Linked to a synthetically derived pan-DR or tetanus-derived epitope Phase I studies currently ongoing
Modified vaccinia virus ankara (MVA) "triplex" vaccine	Vectored delivery by attenuated poxvirus, MVA Triplex vaccine: pp65, IE1/Exon 4, IE2/Exon 5 Favorable safety profile, T cell responses noted in CMV-seronegative recipients in phase I study Vaccine under development at City of Hope, Duarte, California
gB/pp65 lymphocytic choriomeningitis virus (LCMV)-vectored bivalent vaccine	Vectored vaccine designed using LCMV backbone LCMV GP gene replaced by gB, pp65 Disabled, single round of replication No antivector immunity (allows for boosting) Virus-neutralizing antibody, cellular responses
gB/pp65/IE1 trivalent DNA vaccine; gB/pp65 bivalent DNA vaccine	DNA adjuvanted with poloxamer adjuvant and benzalkonium chloride Both bivalent and trivalent vaccines evaluated in phase I studies Impact on CMV disease in HSCT recipients demonstrated in phase II study with bivalent gB/pp65 vaccine Phase III study of bivalent vaccine ongoing Trivalent vaccine evaluated by coadministration with Towne vaccine in prime-boost vaccination
Enveloped virus-like particle (eVLP) vaccine	eVLPs formed by cotransfection of murine Moloney leukemia virus gag and CMV gB constructs Expressed gB extracellular domain fused with transmembrane and cytoplasmic domains of vesicular stomatitis virus G protein Positive immunogenicity and safety profile in phase I studies Currently being developed by Variation Biotechnologies Vaccines Incorporated (VBI)

(Continued on next page)

TABLE 1 (Continued)

Category	Characteristics
CMV vaccines in preclinical development	
Dense body vaccines	Noninfectious Humoral and cellular immune response in preclinical testing Contain gB, pp65, other envelope and tegument proteins
RNA vaccines	Self-amplifying mRNA vaccines Strong antibody and cell-mediated immune responses Platform currently under development by GSK Vaccines and Moderna Vaccines
Electroporated DNA vaccines	DNA plasmid vaccines coadministered with electrical stimulation (SynCon platform) Excellent immunogenicity in preclinical testing Platform under development by Inovio Vaccines
RedBiotech gB/pp65 VLP vaccine	Engineered using recombinant baculovirus Generation of virus-like replicon particles (VRPs) Phase I clinical trial recently initiated Virus-neutralizing antibody and cell-mediated immune responses
Soluble PC vaccine	Currently being developed by Pfizer Vaccines Soluble adjuvanted pentameric complex vaccine Purified from CHO cells Potent, sustained neutralizing antibody responses in mice Developed by Humabs Biomed
MVA-vectored PC vaccine	Based on CMV PC Induces ELISA and neutralizing antibodies in mice Antibodies capable of blocking CMV infection of fetal placental macrophages (Hofbauer cells)
MVA-vectored pp65/IE1 fusion protein	Designated MVA-syn65_IE1 Expands pp65- and IE1-specific T cells derived from CMV-seropositive donors Induces CMV pp65- and IE1 epitope-specific T cells in HLA-transgenic mice
Adenovirus-vectored gB/polyepitope (Ad-gBCMV polyvaccine)	Based on a replication-deficient adenovirus Encodes a truncated form of CMV-encoded gB antigen and multiple CMV T-cell epitopes from eight different CMV antigens as a single fusion protein Immunogenic in preclinical studies in HLA-A2 transgenic mice

^aELISA, enzyme-linked immunosorbent assay; rhIL-12, recombinant human interleukin-12.

that could have important implications for vaccine-mediated protection against recurrent CMV infections during pregnancy (with subsequent congenital transmission) in seropositives (48). Another gB/MF59 vaccine study in young women was recently reported in healthy, CMV-seronegative adolescents (49). Although there was a trend observed that suggested a positive impact of the three-vaccination series on the incidence of CMV infection in the vaccine group compared to placebo, this difference was not significant, likely because the unexpectedly lower incidence of infection in controls than that which had been previously observed in similar studies did not allow discernment of statistical significance. Finally, the gB/MF59 vaccine, evaluated in solid-organ transplant (SOT) recipients, was associated with both a reduction in viremia and in the total number of days of ganciclovir treatment in vaccine recipients compared to those who received placebo. The benefit of vaccination was most striking in CMV-seronegative recipients of transplants from CMV-seropositive donors, and the duration of viremia posttransplantation was inversely correlated with the magnitude of the gB antibody response (50). The future product development plan for the gB/MF59 vaccine, either for the adolescent/young adult population or for transplant recipients, is at this time unclear.

Another recombinant, subunit gB vaccine, GSK1492903A, is expressed in CHO cells as a chimeric protein, with CMV gB sequences fused to a herpes simplex virus 1 (HSV-1) gD sequence. This modification of the gB coding sequence was found to improve overall expression and facilitate purification of the recombinant protein. This platform has been developed by GlaxoSmithKline (GSK) Vaccines. This subunit gB vaccine has been evaluated in a phase I study. The study evaluated a three-dose series (15 μ g/dose) of recombinant gB administered with GSK's AS01 adjuvant in CMV-seronegative volunteers. No serious adverse effects were reported in any of the vaccine recipients. Although results from that study have not yet been published, robust antibody responses, including virus-neutralizing responses, were observed in

vaccinees. These data are publicly available (see www.gsk-clinicalstudyregister.com/study/108890#rs and www.gsk-clinicalstudyregister.com/study/115429#rs).

eVLP VACCINES

Enveloped virus-like particles (eVLPs) are protein structures that mimic wild-type viruses but do not have a viral genome, creating, in principle, a safer vaccine candidate than that engendered by a live attenuated platform. An eVLP vaccine, manufactured by VBI Laboratories (Table 1) and expressing CMV gB, is currently in phase I studies in CMV-seronegative individuals. CMV gB is expressed in this construct as a fusion protein expressed in frame with the transmembrane and cytoplasmic domains of vesicular stomatitis virus (VSV) G protein, a strategy that optimizes immunogenicity (51). A phase I study of this vaccine, VBI-1501A, was initiated in early 2016 and has completed enrollment (<https://clinicaltrials.gov/ct2/show/NCT02826798>). This study compared the safety and immunogenicity of four dose formulations of the gB vaccine, administered with and without an alum adjuvant, in a group of approximately 125 CMV-seronegative volunteers. The preliminary immunogenicity and safety data generated from this study have recently been presented in abstract format (https://www.isvcongress.org/images/downloads/2017_isv_program.pdf). An additional eVLP CMV vaccine candidate, targeting both the gB and pp65 antigens, has also been developed by VBI and has been proposed for use as a therapeutic vaccine for CMV-associated glioblastoma multiforme, to be potentially administered in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) (52).

Another candidate eVLP vaccine against CMV was developed by Redvax GmbH, a derivative of Redbiotec AG. In contrast to the VBI approach, which uses mammalian (HEK) cells to engender the VLP, the Redbiotec expression platform is based on a baculovirus expression system (53). The original patent claim covered potential generation of VLP vaccine candidates containing various combinations of CMV gB, the PC proteins, and glycoproteins gM and gN. The Redvax technology has recently been licensed to Pfizer Vaccines; the product development plan and timeline for phase I studies have not been announced, although a PC vaccine is the leading candidate.

VECTORED CMV VACCINES

There are several vectored CMV vaccines that have been evaluated in phase I and phase II studies. This vaccine approach employs a heterologous viral vector to deliver CMV-encoded immunogens, typically some combination of gB, pp65 (UL83), and IE1. Two vaccines have utilized an attenuated canarypox vector to deliver either gB (ALVAC-CMV [vCP139]) or pp65 (ALVAC-CMV [vCP260]) subunit (54, 55). Since the canarypox vector cannot complete its replicative cycle in mammalian cells, this vectored approach was, as predicted, found to be highly attenuated when administered to human volunteers. However, the ALVAC-CMV [vCP139] vaccine unfortunately failed to increase virus-neutralizing titers among seropositive recipients and it did not induce significant neutralizing titers in seronegative subjects. Therefore, it was next evaluated in a "prime-boost" strategy in which priming with ALVAC-CMV [vCP139] was followed by administration of either live attenuated Towne vaccine (described below) or gB/MF59 subunit (54, 56). The prime-boost approach using ALVAC-CMV [vCP139] and Towne induced neutralizing antibody responses comparable to those seen with natural CMV infection, but there was no benefit derived from combining the ALVAC vaccine with gB/MF59.

With respect to the ALVAC-pp65 vaccine, phase I studies of ALVAC-CMV [vCP260] demonstrated a favorable safety profile as well as robust induction of pp65-specific cytotoxic T lymphocyte (CTL) and antibody responses in healthy, CMV-seronegative adults (55). This vector was remarkably potent at inducing CTL responses in seronegative volunteers after two doses (55). A phase II study of this vaccine in the hematopoietic stem cell transplant (HSCT) patient population (<http://www.clinicaltrials.gov>

registration no. NCT00353977) was completed in 2008, but results have yet to be published.

Another vectored approach utilized in design of a CMV vaccine was based on use of an alphavirus vector, Venezuelan equine encephalitis (VEE) virus. In this strategy, genes encoding VEE structural proteins were replaced with genes expressing the extracellular domain of gB and a pp65-IE1 fusion protein in a double-promoter replicon (57, 58). Progeny VLPs generated using this strategy were replication deficient but supported abundant recombinant protein expression and were highly immunogenic. In a phase I study in CMV-seronegative volunteers, vaccinees received either three low doses (1×10^7 infectious units) or three high doses (1×10^8 infectious units) of the vaccine, by either subcutaneous or intramuscular injection, over a 24-week period. Participants tolerated the vaccine well and developed CTL and neutralizing antibody responses to all three CMV antigens (59). This platform has also been used to develop VLP vaccines targeting the gH/gL and PC proteins, and these vaccines have demonstrated immunogenicity in mouse studies (60, 61). This platform was originally developed by AlphaVax Vaccines, which was acquired by Novartis Corporation in 2008, and is now held by GSK following their purchase of Novartis Vaccines. Plans for future studies are unknown at this time.

A more recent innovation in vectored CMV subunit vaccine design has employed the attenuated recombinant lymphocytic choriomeningitis virus (LCMV) platform (62). This vector utilizes producer cells that constitutively express the LCMV viral glycoprotein (GP), making it possible to replace the gene encoding LCMV GP with vaccine antigens of interest in plasmid constructs delivered in *trans* to these producer cells. The resulting rLCMV vaccines are replication defective but elicit CTL and CD4⁺ T cell responses as well as neutralizing antibody responses. A theoretical advantage of these vaccines relevant to CMV is the observation that they do not elicit vector-specific antibody immunity, allowing readministration of booster vaccines. This feature might be highly desirable for sustaining protection (conferred by periodic booster immunizations) against congenital CMV in women of reproductive age during serial pregnancies. The utility of this approach was recently demonstrated in the GPCMV model of congenital infection (63, 64), providing support for evaluation of these vectors in human clinical trials. This platform was developed by Hookipa Biotech AG. The company is currently evaluating a replication-defective LCMV-vectored CMV vaccine, designated HB-101, which is a bivalent vaccine containing two vectors expressing pp65 protein and gB, respectively. A placebo-controlled, phase I dose escalation study has recently been completed comparing three different doses of vaccine (<https://clinicaltrials.gov> registration no. NCT02798692) in a three-vaccination regimen, with vaccine administered at 0, 1, and 4 months by the intramuscular route. Preliminary data have been reported that indicate that vaccinees developed virus-binding and neutralization antibody responses. Vaccine recipients also demonstrated interferon gamma enzyme-linked immunosorbent spot (IFN- γ ELISPOT) assay responses specific for gB and pp65.

Other virally vectored CMV vaccines have been explored in preclinical studies and, in one instance, an ongoing phase II study. The vector in this instance is modified vaccinia virus Ankara (MVA; study described in more detail below). MVA has been used to express a variety of CMV antigens, including pp65, gB, IE1, IE2, and the PC proteins. In rodent and nonhuman primate model systems, MVA-vectored vaccines have demonstrated excellent immunogenicity (65–67). In the GPCMV congenital infection model, MVA-vectored gB/pp65 (GP83) homolog-based vaccines were immunogenic and protective against congenital transmission and disease (68). In mice or macaques vaccinated with MVA-vectored PC vaccine(s), neutralizing antibody responses were engendered that blocked CMV infection of Hofbauer macrophages, which are fetus-derived cells localized within the placenta—an observation of particular relevance to a vaccine aimed at preventing congenital CMV transmission (69). A pp65/IE1 fusion protein has been expressed in MVA, and the resulting recombinant, designated MVA-syn65_IE1, has been shown to activate and expand the levels of pp65- and IE1-specific T cells

derived from CMV-seropositive donors following infection of CD40-activated B cells and to induce CMV pp65- and IE1-epitope-specific T cell responses in HLA-transgenic mice (70). A triplex MVA-vectored vaccine is currently in a phase II trial in HSCT patients, employing a placebo-controlled, two-dose study design in which the vaccine is delivered by the intramuscular route (<https://clinicaltrials.gov> registration no. NCT02506933). The vaccine encodes immunogenic peptides corresponding to pp65, IE1, and IE2 (71). The primary endpoints for this study include CMV reactivation, CMV disease, and use of antiviral therapy in an HSCT population. Although this information will be useful in efforts to develop a CMV vaccine for the HSCT population, the relevance of this MVA vaccine to the problem of protection against acquisition of CMV infection in women, or for prevention of congenital CMV infection, is less clear.

NUCLEIC ACID-BASED CMV VACCINES

Nucleic acid-based vaccines represent another emerging platform for immunization against CMV infection. The clinical trials performed to date have, for the most part, been conducted in the HSCT and SOT patient populations, with the goal of reducing CMV disease in this uniquely vulnerable population. ASP0113 (previously known as VCL-CB01 and TransVax) is a DNA-based CMV vaccine targeting both gB and pp65. It was first developed by Vical Corporation and is currently under license to Astellas for phase III clinical trials (described below) and commercialization. It consists of two plasmids, VCL-6368 and VCL-6365, formulated with poloxamer CRL1005 and a cationic surfactant, benzalkonium chloride (72, 73). VCL-6368 encodes a pp65 protein which contains a modification in the protein kinase domain spanning amino acids 435 to 438. VCL-6365 encodes the extracellular domain (amino acids 1 to 713) of CMV gB, derived from the AD169 strain sequence.

A phase I clinical trial evaluating the safety of ASP0113 found no serious adverse events in the 22 CMV-seropositive and 22 seronegative individuals vaccinated (73). The seronegative subjects engendered pp65 and/or gB-specific T cell responses and gB antibody responses, whereas the members of the seropositive vaccinated groups showed increases only in pp65-specific T cell responses. Subsequent evaluation of ASP0113 efficacy in a double-blind, placebo-controlled phase II trial in CMV-seropositive HSCT patients (<http://www.clinicaltrials.gov> registration no. NCT00285259) demonstrated an acceptable safety profile and a statistically significant reduction of CMV viremia following vaccination, as well as a trend toward reduced use of anti-CMV antivirals in immunized subjects (74). Safety was further evaluated in an open-label, uncontrolled phase II study (75). A phase III clinical trial was recently initiated to continue the evaluation of ASP0113 efficacy in HSCT patients (<http://www.clinicaltrials.gov> registration no. NCT01877655). In light of the relatively low responses to gB induced by this vaccine, efficacy may depend upon the ability of the anti-pp65 responses to suppress CMV reactivation. Studies in SOT patients (phase II, <http://www.clinicaltrials.gov> registration no. NCT01974206) and dialysis patients (phase I, <http://www.clinicaltrials.gov> registration no. NCT02103426) have also been undertaken.

For the most part, these platforms have focused on the transplant population, and the relevance of these DNA vaccines to prevention of congenital CMV remains to be defined. However, a nonadjuvanted, trivalent DNA vaccine, VCL-CT02, has been developed with nontransplant indications in mind. This vaccine includes the T cell target IE1 in addition to the gB and pp65 coding sequences. VCL-CT02 has been evaluated in phase I clinical trials (<http://www.clinicaltrials.gov> registration no. NCT00370006 and NCT00373412), and Vical has proposed further development of the trivalent DNA vaccine as a candidate for immunization against congenital CMV infection (76). Vical has also recently published results from preclinical evaluation of gB and pp65 plasmids delivered in combination with an improved adjuvant system, the cationic lipid-based adjuvant Vaxfectin, which has been observed to increase the immunogenicity of antigens in animal models (77, 78).

An alternative nucleic acid-based vaccine, based on the proprietary SynCon tech-

nology described by Inovio Pharmaceuticals, has been developed and evaluated in preclinical studies. The Inovio technology involves computational analysis of the sequences of several common strains or variants of vaccine antigens of interest, followed by assembly of a consensus gene sequence synthetically created for the antigen which is then inserted into a DNA plasmid for further evaluation, including immune responses (79). An additional innovation is the use of electroporation (80, 81), in the context of either the intradermal or intramuscular route of administration, to increase DNA uptake and, hence, vaccine effectiveness. The DNA electroporation strategy has been explored in the murine CMV (MCMV) model (82). Using this strategy, immunization of mice with the murine CMV gB homolog M55 induced immune responses that provided modest protection against lethal MCMV challenge. Other immunogens, such as gene products encoded by M84, m04, or IE1, appeared to be even more effective than M55. The pipeline for clinical trials designed to evaluate this platform in clinical trials has not yet been defined, but the recent initiation of phase I studies of a SynCon Zika virus DNA vaccine (<https://clinicaltrials.gov> registration no. NCT02809443) suggests that this approach may be studied for CMV in the near future. More recently, preclinical evaluation of this platform using human CMV-specific constructs has been reported following studies in C57BL/6 mice, and this vaccine approach was found to evoke a high degree of T cell immunogenicity, particularly for the gH/gL vaccine candidate (83, 84).

RNA-based nucleic acid vaccines against CMV have also been developed and explored in preclinical studies. Mouse studies demonstrated that RNA expressed from bicistronic alphavirus-derived replicon particles elicited neutralizing antibody responses to the gH/gL complex and that these antibodies cross-neutralized diverse clinical isolates of CMV (60). A self-amplifying mRNA vaccine platform developed by Novartis (now GSK) Vaccines (85, 86) was evaluated in rhesus macaques. The vaccine contained gB and a pp65-IE1 fusion construct, and the two self-amplifying RNAs were formulated separately with a cationic nanoemulsion and administered by the intramuscular route in animals at a dose of 75 μ g of RNA for each antigen. Antigen-specific immune responses, including both total anti-gB IgG and neutralizing antibody responses, were detected in all animals ($n = 6$) after a single immunization and were boosted 3-fold after a second immunization. After two immunizations, all animals also had measurable CD4⁺ and CD8⁺ T cell responses. The product development plan for this vaccine has not been announced. Another RNA vaccine platform based on self-amplifying mRNA, developed by Moderna Therapeutics, has been described. In this approach, synthetic mRNAs are formulated with lipid nanoparticles to enhance processing and immunogenicity. There has been a phase I study of an mRNA-based influenza vaccine developed using this technology, and the vaccine was shown to be safe and highly immunogenic in 23 volunteers immunized in the context of an ongoing double-blind, placebo-controlled, dose-escalating trial (87). Moderna Vaccines has recently announced that preclinical development of a six-component CMV mRNA vaccine, mRNA-1647, will commence. It will consist of the 5 PC constituents and gB, although a detailed product development plan has not yet been announced.

LIVE ATTENUATED AND “DISC” VACCINES

Although the time-honored approach of serial tissue culture passage (aimed at attenuation of virus) represents the first and oldest technique used in the attempt to develop a CMV vaccine, recent years have seen this approach fall into disfavor, perhaps driven by the concern that any live virus vaccine generated in this fashion, no matter how otherwise attenuated it might be, could carry with it an unacceptable level of risk of establishing latency and/or undergoing productive replication in the immunized host. The first live virus vaccines for CMV were based on the laboratory-adapted strains AD169 and Towne (88). The molecular basis for attenuation of these strains following cell culture passage was for many years largely unknown. However, the use of improved molecular methods to characterize these viruses since their original isolation and serial passage has revealed that, among the many genetic changes acquired during cell culture passage, both strains acquired mutations abrogating proper expression of

the PC. The Towne strain contains a 2-bp insertion (TT) leading to a frameshift mutation in UL130, and the AD169 strain has a 1-bp insertion (A) generating a frameshift mutation in UL131 (89, 90). These mutations are believed to have contributed to their attenuation, but probably at the expense of their immunogenicity, particularly with respect to induction of epithelial- and endothelial-cell neutralizing antibodies. Undoubtedly, other recently identified genetic changes have also likely impaired the immunogenicity of these viruses (particularly for the Towne strain, described in more detail below) in the vaccinated host, in addition to the impact of the aforementioned mutations on generation of anti-PC antibodies.

The mutation in the UL130 coding sequence (as well as other attenuating mutations) notwithstanding, Towne vaccination was observed to provide some (85%) protection against severe CMV disease in SOT recipients, though it did not protect against infection (91–93). Towne vaccine also failed to protect young women against acquisition of CMV infection from their toddlers in group day care attendance, whereas preexisting immunity to CMV did protect these mothers from recurrent infection (94). The apparent lack of efficacy of the Towne vaccine (compared to “naturally acquired” immunity) in this study was attributed to suboptimal immunogenicity of a particular lot of vaccine virus, and was suggested by suboptimal induction of neutralizing antibody following immunization. In another, later study of Towne vaccine, an effort was made to improve Towne’s immunogenicity through the coadministration of recombinant interleukin-12 (IL-12) (95), but although the inclusion of IL-12 resulted in improved antibody and cell-mediated responses, this approach no longer appears to be in clinical development.

More recent studies of live attenuated CMV vaccines have attempted to improve on the immunogenicity of Towne, by generation of “chimeric” viruses containing genomic segments from the Towne strain and from a less attenuated CMV isolate, the Toledo strain. These Towne/Toledo chimeric vaccines, generated by cotransfection of overlapping cosmid libraries (96–98), were initially evaluated in a phase I trial in CMV-seropositive subjects (99). The goal was to identify a recombinant that was attenuated relative to the Toledo strain (which demonstrated the capacity to proceed CMV disease in challenge studies in volunteers) but that was more immunogenic (and by inference, potentially more protective) than the Towne vaccine. In that phase I study in CMV seropositives, transaminase levels and leukocyte counts were compared among the chimera recipients and against historical control data from studies where volunteers were experimentally inoculated with the Toledo strain (99). These comparisons suggested that all of the Towne/Toledo chimeras were attenuated relative to the parental Toledo strain.

These vaccines were next evaluated for safety and immunogenicity in CMV-seronegative recipients in a dose-range study (10^1 to 10^3 PFU/dose; <http://www.clinicaltrials.gov> registration no. NCT01195571). No vaccinee in either study demonstrated any viral shedding of the vaccine strains. Eleven of 36 CMV-seronegative men enrolled in the seronegative study underwent seroconversion; that result was more common in those inoculated with chimera candidates 2 and 4 (100). Study participants that had seroconverted had demonstrable levels of neutralizing antibody, and some demonstrated CD8⁺ T cell responses to IE1. Notably, since the time that these studies were initiated, more-detailed sequence characterization of the chimeras has revealed that all of the chimera vaccines carried a disrupted copy of the *UL128* gene, derived from the parent Toledo strain. Therefore, like the AD169 and Towne vaccines, the Towne-Toledo chimeras are incapable of assembling a wild-type PC and, as such, would be predicted to likely be incapable of eliciting PC-specific neutralizing antibodies in vaccinees. What impact this would have on the potential efficacy of a congenital CMV vaccine is unclear. The recently reported DNA sequence analysis of the Towne-Toledo chimeras should aid in elucidating the molecular basis for the observed differences in immunogenicity seen in this clinical trial, as well as in identifying potential genetic markers conferring attenuation (101).

In light of persistent and incompletely resolved concerns about the safety profile of

live attenuated CMV vaccines, the generation of transgenic disabled infectious single-cycle (DISC) vaccines is an attractive alternative. Such vaccines could, in principle, elicit a full repertoire of antibody responses to envelope glycoproteins, including the PC, and could induce a broad range of T cell responses to multiple viral proteins, providing a much greater breadth of responses than those induced by pp65 and IE1 subunit vaccines. One such recently developed CMV DISC vaccine, V160, is currently undergoing phase I clinical trials in both seronegative and seropositive subjects. This vaccine, designed by Merck Vaccines, has a restored, wild-type PC sequence in which the frameshift mutation in the first exon of UL131—a mutation that underlies the epithelial tropism deficiency in AD169 resulting from abrogation of proper assembly of the PC—was repaired in *Escherichia coli* by recombineering of an infectious bacterial artificial chromosome (BAC) clone of the AD169 genome, followed by recovery of repaired virus harvested after transfection of BAC DNA onto human retinal pigmented epithelial (ARPE-19) cells. V160 was further modified such that viral proteins IE1/IE2 and UL51 were expressed as fusion proteins with FKBP12, a rapamycin-binding protein (102–104). Since UL51 and IE1/2 are essential for replication competence (105, 106), V160 is able to propagate in ARPE-19 cells only in the presence of a synthetic stabilizing ligand, Shield-1. Since Shield-1 is not found in nature, the fusion protein is rapidly degraded and viral replication is inhibited in any immunized subject (103, 104), providing an excellent safety profile for the vaccine.

V160 has recently completed phase I testing. The vaccine and a placebo were compared in a low-, intermediate-, and high-dose comparison study, in both CMV-seronegative and -seropositive individuals, with or without Merck aluminum phosphate adjuvant (MAPA). These data have not been published but were recently reported (107). V160 combined with MAPA adjuvant induced neutralizing antibodies after 3 doses at 0, 1, and 6 months. The neutralizing levels measured in epithelial cells demonstrated titers equal to or higher than those observed in naturally seropositive subjects. The vaccine also induced interferon gamma-producing T cells as measured by ELISPOT assays (following stimulation performed *in vitro* with CMV peptides) at levels equal to or higher than those seen with natural seropositives. Intradermal vaccination also gave good responses. The vaccine was well tolerated in this phase I study, and there was no virus shedding in inoculated subjects. Merck plans to proceed to evaluate this candidate vaccine in a phase II study.

PEPTIDE VACCINES

Other CMV vaccines currently in clinical trials include a number of peptide-based approaches. These vaccine candidates are generally focused on strategies aimed at providing protection of HSCT recipients against development of CMV disease post-transplant, so their relevance to prevention of congenital CMV infection is not currently clear. It is known that pp65-specific CTL responses are associated with protection of HSCT patients from CMV disease, and this observation has helped to drive development of pp65-based peptide vaccines. CMV pp65 epitopes critical in protection have been fused to either a synthetic pan-DR epitope (PADRE) or a natural tetanus (Tet) sequence and have been evaluated in phase I trials (<http://clinicaltrials.gov> registration no. NCT00722839), with and without synthetic CpG Toll-like receptor 9 (TLR9) agonist adjuvant 7909 (108, 109). This adjuvant, when administered with PADRE and Tet pp65, enhanced immune responses in vaccinees (109). One particular pp65 epitope that has been examined in detail, pp65 495-503, is estimated to cover approximately 35% of the U.S. population, based on the overall frequency of the HLA A*0201 allele. Thus, it is conceivable that a polypeptide vaccine spanning a sufficient number of T cell epitopes could be effectively employed as a population-based vaccine against congenital CMV. Phase II studies of the Tet-pp65 vaccine, administered with TL9 adjuvant 7909 and designated CMVpp65-A*0201 or CMVPepVax (110), are now in progress (<http://clinicaltrials.gov> registration no. NCT02396134), with enrollment targeting HLA-A*0201-positive, CMV-seropositive HSCT recipients.

CMV VACCINES IN PRECLINICAL DEVELOPMENT

Although they have not yet entered clinical trials, dense bodies (DBs) are being explored as a novel CMV vaccine candidate (111). DBs are fully enveloped particles formed following CMV infection in cell culture, and although they are completely devoid of viral DNA, they contain many viral glycoproteins and tegument proteins that are targets of the immune response. As such, they are noninfectious, but capable of eliciting humoral and cellular responses to multiple CMV-encoded structural proteins, making them an intriguing vaccine candidate. Preclinical studies in mice demonstrated that DBs, when administered as a vaccine, induced consistent neutralizing antibody titers and cellular immune responses across multiple animal experiments and various preparation methods and in the absence of viral gene expression (112, 113). In preclinical studies, immune responses did not depend upon the presence of adjuvant in the vaccine formulation (114). The antibody response resulting from vaccination with these DBs was also shown to prevent infection of both fibroblasts and epithelial cells by the clinical CMV isolate VR1814 in cell culture (112). Methods to scale up the processes of manufacture and purification have been previously described (115). The Serum Institute of India has licensed the technology for production of a DB vaccine, designated CAP CMV001, from Vakzine Projekt Management GmbH. Additional plans for clinical development of this vaccine have not been announced.

A soluble, adjuvanted CHO cell-expressed PC vaccine has also been described. Humabs Biomed has reported that, in preclinical studies, immunization of mice with this version of a PC vaccine (formulated with several different candidate adjuvants) elicited neutralizing antibody titers that persisted at high levels over time and that were much more potent than those observed in CMV-seropositive individuals (116).

Other novel approaches that have been evaluated only in animal models to date have explored the possibility of either modifying or targeting CMV-encoded immune modulation genes in vaccine design. Strategies have included a vaccination approach targeting virally encoded IL-10 in the RhCMV model (117) and the design of live attenuated vaccines with engineered deletions of CMV-encoded protein kinase R evasins in the GPCMV model (118). Interestingly, the use of live attenuated CMV itself as a vector for expressing vaccine targets for other pathogens, such as HIV-1, has been developed and tested in nonhuman primate challenge models using simian immunodeficiency virus (SIV), with surprising and impressive levels of effectiveness in vaccine-mediated clearance of pathogenic SIV infection in the macaque model. Protection in the macaque SIV model was mediated in part through induction by the RhCMV-vectorized SIV vaccine of a strong and nonconventional effector CD8⁺ T cell response (119, 120). The human CMV versions of these vectors are now being developed for clinical trials for HIV vaccines. If safety endpoints are met, these vectors could change the landscape for design of vaccines not only against CMV and HIV, but also against potentially many other pathogens, for which key protective antigens could be vectored by a recombinant CMV backbone.

Another vectored vaccine, Ad-gBCMVPoly, is a novel CMV vaccine candidate based on a replication-deficient adenovirus. This platform has been developed by the Queensland Institute (121). This vaccine encodes a truncated form of gB antigen and multiple CMV T cell epitopes from eight different CMV antigens. The peptide sequences are restricted through HLA class I and class II alleles and expressed as a single fusion protein. The vaccine has demonstrated immunogenicity in HLA-A2 transgenic mice.

TARGET POPULATION FOR DEPLOYMENT OF A CMV VACCINE

As noted earlier in this minireview, the NAM report (14) modeled the hypothetical deployment of a CMV vaccine based on the presumption that it would be administered to 12-year-old boys and girls. Certainly, if the goal of a CMV vaccine program is to prevent congenital CMV infection, immunization of the preadolescent population would in principle be desirable, both to prevent sexual transmission of CMV in young adults and to block vertical transmission from mother to fetus as adolescent women

TABLE 2 Potential target populations for eventual deployment of a licensed CMV vaccine**Objective and target population for CMV vaccination**

Universal vaccination of 12-year-old boys and girls
Vaccination of seronegative women of child-bearing age
Vaccination of seropositive women of child-bearing age (prevention of recurrent infection)
Universal immunization of all infants
Vaccination of SOT and HSCT recipients
Vaccination of HSCT donors

enter their child-bearing years. Such a strategy could be readily incorporated into adolescent meningococcal/acellular pertussis/human papillomavirus immunization programs. Alternative approaches could include targeting seronegative women of child-bearing age for vaccination, based on the evidence that primary infections during pregnancy are the most disabling to the developing fetus, or providing universal immunization of all women of child-bearing age irrespective of CMV serostatus, based on the premise that recurrent infections with resultant disabling congenital transmission can occur in women with preconception immunity, and that vaccination of these women could limit the potential for such transmission events. There is likely value in a CMV vaccine capable of both priming naive individuals and boosting relevant (protective) immunity in CMV-seropositive individuals. Of course, a strategy that included immunization of seropositives would require a CMV vaccine that was capable of boosting natural immunity, as has been demonstrated with the gB/MF59 vaccine (48).

Universal vaccination of all infants/toddlers is another strategy that has been advocated. This approach could block transmission of virus among children attending group day care; this strategy, in turn, could prevent transmission of CMV from toddlers to their pregnant mothers. It has been noted that the force of infection of CMV is sufficiently low that even a modestly effective vaccine, such as the gB/MF59 vaccine (46), would likely have a substantial impact on the circulation of CMV in the human population (122). Thus, a vaccine strategy targeting elimination of CMV infection based on universal immunization of young children and/or adolescents is worth considering, and surveys have indicated that a safe vaccine at a reasonable cost would be widely accepted (123).

Vaccination against CMV is also a laudable goal for the SOT and HSCT populations. Although several of the vaccines discussed in this minireview are designed with this patient population in mind, it is also quite feasible that a highly effective vaccine licensed for the transplant population could, once it becomes available, be employed by primary care practitioners as a prophylactic intervention against congenital CMV infection. Prevention of CMV in transplant recipients by vaccination has had some early successes and is being pursued by several manufacturers, and lessons learned from these studies should be applied to the problem of congenital CMV.

A summary of the patient populations that potentially could be targeted for eventual deployment of a licensed CMV vaccine is provided in Table 2. For prevention of congenital CMV, we assert that, at a minimum, a vaccine for seronegative women in the United States and Europe is needed. Such a vaccine would be valuable and would have an important impact on child health, even if prevention of transmission in seropositives is not completely realized. This assertion is based on the different consequences of congenital CMV in women with primary infection during pregnancy compared to those with recurrent infection. Given the higher likelihood of the presence of substantial percentages of seronegative women of child-bearing age in the United States and Europe, plus the demonstration that natural seropositivity is partially protective, such a vaccine strategy should be deployed as soon as possible, even as the approach to vaccination of seropositives continues to be studied.

TABLE 3 Strategies for demonstration of effectiveness of a CMV vaccine in clinical trials prelicensure

Prelicensure demonstration of CMV vaccine efficacy
Protection against experimental challenge (e.g., Toledo challenge studies)
Protection of toddlers against acquisition of CMV infection in group day care
Protection of mothers (parents?) against acquisition of CMV infection from their children attending group day care
Protection of SOT/HSCT patients against acquisition of CMV/reactivation/CMV DNAemia/antiviral therapy/complications of transplantation
Protection of adolescents/young adults against acquisition of CMV infection
Cohort study in women prior to establishment of pregnancy for protection against: <ul style="list-style-type: none"> Congenital CMV infections Congenital CMV-associated disease in infants
Combination of human clinical trial data with animal studies of vaccine protection against congenital CMV transmission (FDA Animal Rule)

CHALLENGES TO LICENSURE OF A CMV VACCINE: WHAT WILL IT TAKE TO GET THERE?

Many challenges exist that have hampered development and licensure of a CMV vaccine. As already discussed, uncertainty remains about the precise correlate(s) of protective immunity for the placenta and developing fetus, although a vaccine that elicits high levels of antibody to the PC and to gB (of sufficient magnitude that cell entry of virus is prevented) is very likely to be successful. There is continued discussion about what kind of study design and efficacy endpoints would be required for approval of a vaccine against congenital CMV infection, with particular interest in defining the necessary size of the potential clinical trial(s) that would be necessary for licensure. It has been noted that a phase III trial would require over 50,000 subjects, assuming a vaccine efficacy of 50%, to demonstrate a statistically significant reduction in CMV disease in the newborn as the major experimental endpoint (124). Given the overwhelming expense and logistic challenges of such a study, alternative approaches to experimentally demonstrate CMV vaccine efficacy prior to licensure are required. Some of these potential study designs are summarized in Table 3. These could include studies aimed at demonstrating prevention of CMV disease in SOT and HSCT patients, and/or reduction in horizontal transmission of CMV infection in infants or toddlers (particularly those in attendance in group daycare). The endpoint of preventing virus acquisition in seronegative women of child-bearing age which has previously been employed in CMV vaccine clinical trials may set the bar too high and may result in exclusion of vaccine candidates that could have effectively reduced congenital transmission, or at the least reduced CMV-associated disease in the newborn. Importantly, the licensure pathway may need to combine results of human clinical studies with evidence of protection against congenital transmission in animal models (acknowledging that the species specificity of CMVs largely precludes testing of human CMV in animals). This licensure strategy evokes the Food and Drug Administration (FDA) "Animal Rule" (<https://www.fda.gov/downloads/drugs/guidances/ucm399217.pdf>), which can be applied in a situation when human efficacy trials are not considered to be either feasible or ethical.

A consensus statement about the optimal prelicensure study for a potential CMV vaccine emerged as a result of a meeting in 2012 where the FDA commissioned a symposium with representatives from government, industry, academia, and parent groups. These stakeholders met to discuss challenges presented by congenital CMV infection and the current state of vaccines in development and assessed the impact of congenital CMV (https://www.accessdata.fda.gov/scripts/fdatrack/view/track_project.cfm?program=cber&id=CBER-OVRR-Cytomegalovirus-Vaccine-Workshop). Different CMV vaccines in preclinical development, clinical trial design, and high-priority areas for future research were reviewed at the FDA workshop. A consensus emerged that pivotal clinical trials of CMV vaccines should be powered to demonstrate protection against congenital CMV transmission as the key prelicensure endpoint (125). Prevention of congenital infection was considered to be a more accessible study endpoint than

prevention of congenital CMV-associated disease—which is evident only rarely at birth and may take years to become clinically manifest, particularly in the setting of sequelae such as delayed-onset SNHL. However, although we agree that this is the ideal study endpoint, consideration of other strategies, including those outlined in Table 3, should continue to be contemplated and discussed.

Finally, a significant challenge to achieving the goal of a vaccine against congenital CMV infection is the assertion that we know too little about correlates of protection and the impact—or lack thereof—of maternal immunity to justify moving forward with clinical trials. The history of vaccinology is replete with examples of licensed vaccines initially employed to confront urgent public health problems that were eventually replaced with improved, enhanced products. Although much remains to be learned about the optimal vaccine strategy for prevention of congenital CMV, the urgency of this public health problem demands action. As Voltaire noted in 1772 in the poem *La Béguéule*, “Dans ses écrits, un sage Italien dit que le mieux est l’ennemi du bien”—the best is the enemy of the good. Promising CMV vaccine candidates demonstrating potential in phase I and II studies should be evaluated in larger efficacy trials, with the goal of expeditious licensure of a vaccine to confront this major cause of injury in newborns, even while work is ongoing to continue to elucidate the key correlates of protection that can inform the design of second-generation vaccines.

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