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A Randomized Trial to Prevent Congenital Cytomegalovirus Infection (CMV)

Protocol

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1 Introduction

1.1 Study Abstract

Cytomegalovirus (CMV) is the most common congenital infection, with a prevalence of approximately 1% in the United States, translating into 44,000 congenitally infected infants per year. A substantial proportion of these 44,000 infants will die or suffer permanent injury as a result of their infection. The severity of congenital infection is greatest with primary maternal CMV infection. Currently, there is no proven method of preventing congenital CMV infection, and the approach to primary maternal CMV infection in the United States is haphazard and ineffective. One small, non-randomized study suggests that maternal administration of CMV hyperimmune globulin may reduce the rate of congenital CMV infection following maternal primary infection. We propose to screen gravidas in the first half of pregnancy for recent primary CMV infection, and evaluate in a proper randomized clinical trial whether maternal administration of CMV hyperimmune globulin will prevent congenital CMV infection.

1.2 Primary Hypothesis

Maternal administration of CMV hyperimmune globulin will lower the rate of congenital CMV infection among the offspring of women who have been diagnosed with primary CMV infection during early pregnancy.

1.3 Purpose of the Study Protocol

This protocol describes the background, design and organization of the randomized clinical trial and may be viewed as a written agreement among the study investigators. The Data and Safety Monitoring Committee (DSMC) and the Network Advisory Board review the protocol. Before recruitment begins, the protocol is approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network Steering Committee, and the Institutional Review Board (IRB) of each clinical center. Any changes to the protocol during the study period require the approval of the Steering Committee and the IRBs; major changes also require the approval of the DSMC.

A manual of operations supplements the protocol with detailed specifications of the study procedures.

2 Background

2.1 Burden of CMV disease

Cytomegalovirus (CMV) is the most common congenital infection. Between 1 and 4% of seronegative pregnant women will seroconvert (have a primary infection) during pregnancy. With a prevalence of approximately 1% in the United States, it is estimated that 44,000 congenitally infected neonates are born annually.[1] Almost 400 children die each year from this disease, and up to 8,000 develop permanent disabilities. Congenital CMV accounts for 20-30% of cases of congenital hearing loss, and is a leading cause of hearing loss in the U.S. Congenital CMV affects more children than other conditions that have routine screening, such as trisomy 21 and spina bifida. Unlike these conditions, however, congenital CMV infection is potentially preventable during the antepartum period.

Following maternal primary CMV infection, approximately 40% of fetuses will acquire CMV infection in utero. Those infected in the first trimester are much more severely affected. A third of congenitally infected neonates are born prematurely. At birth, 10-15% of congenitally infected neonates will have symptoms such as microcephaly, petechiae, hepatomegaly, splenomegaly, jaundice, pneumonitis, encephalitis, seizures and low birth weight. Of these symptomatic infants, 6% will die, mostly from disseminated intravascular coagulation, hepatic dysfunction or bacterial superinfection. Approximately 90% of those with clinically apparent infection at birth will develop sequelae, such as bilateral hearing loss, chorioretinitis or severe developmental delay. The likelihood of survival with normal intellect and hearing after symptomatic congenital CMV infection is small.[1] Of the 85-90% of infants that are asymptomatic at birth, 10-15% will develop permanent disabilities such as hearing loss, optic atrophy, developmental delay, spastic diplegia or quadriplegia by 2 years of age. The estimated annual cost of caring for these children is one to two billion dollars.

Additionally, congenitally infected neonates continue to excrete large quantities of virus in their urine and saliva, for up to 6 years or perhaps longer. This long-term excretion contributes to the spread of infection, thus amplifying the public health burden.

2.2 Potential for Prevention of Congenital Disease

Administration of hyperimmune globulin (HIG) to prevent congenital CMV infection has been successful in an animal model (guinea pig) in reducing both fetal infection as well as fetal effects such as growth restriction. Proposed mechanisms of action include immunomodulatory effects, reduction of viral load, and /or decreased placental inflammation.[2]

Human neonates born to mothers with evidence of prior CMV infection have been shown to be at decreased risk of transfusion-associated CMV infection compared to neonates born to mothers without prior CMV infection, which suggests that maternal CMV antibodies that cross the placenta are protective.[3] As part of a proof of concept study, Nigro and colleagues studied the use of CMV hyperimmune globulin in 84 women who had a primary CMV infection at less than 21 weeks' gestation, or who declined amniocentesis following a primary CMV infection.[4] They were offered monthly HIG (100U/kg) infusions until delivery as preventive therapy, and 37 women accepted therapy. Those who received HIG had a significantly lower rate of congenitally infected neonates (40% vs 16%; P=0.02). Severe symptomatic neonatal infections were noted in 3 of 19 infected infants whose mothers did not receive HIG compared with none of the 6 infected infants whose mothers received HIG (P=NS).[4] While these results are promising, they are not even close to definitive, for the study was neither randomized nor blinded. Accordingly, an accompanying editorial in the *New England Journal of Medicine* called for the conduct of a randomized, prospective, placebo-controlled trial.[5] Revello and colleagues conducted a randomized trial and the final results were recently published in the *New England Journal of Medicine*. The researchers concluded that among the 124 women randomized in the study, "treatment with

hyperimmune globulin did not significantly modify the course of primary CMV infection during pregnancy". However, the study suggested a hint of benefit with a 30% congenital infection rate in the hyperimmune globulin group versus 44% in the placebo group (p= 0.13). The publication also raised a potential safety issue of preterm birth with a 13% rate of preterm delivery, preeclampsia, and fetal growth restriction in the hyperimmune globulin group versus 2% in the placebo group (p= 0.06).[6]

In the past, conducting such a trial was not feasible, due to the difficulty in identifying a primary CMV infection. IgM antibodies are a good indicator of acute or recent infection but cannot always be correlated with a primary infection since they can persist in serum for up to 18 months following primary infection and can reappear during reactivation or reinfection. IgM may also be present due to heterotypical immune responses during intercurrent infections.[7] Thus, documented IgG seroconversion was necessary for determining recent infection. This required serial blood draws to detect seroconversion. More recently, measurement of CMV IgG avidity has proven to be a powerful tool in distinguishing primary from recurrent CMV infection. IgG avidity is the strength with which the IgG attaches to the antigen, and this strength matures with time. IgG produced within the first 3 months following primary infection exhibits low avidity.[8-10] This advance has made accurate serologic testing for primary CMV infection feasible on a large scale. An alternative to secondary prevention of fetal CMV infection includes primary prevention with the use of a maternal CMV vaccine. In the 1990s a vaccine based on CMV envelope glycoprotein B with MF59 adjuvant entered clinical trials. Recently, Pass et al published their phase 2, randomized, placebo-controlled, double-blind trial in which the vaccine was tested on healthy women who had delivered a baby within the past year.[11] In this study, more than 18,000 women were screened to identify 4,509 seronegative subjects, of whom only 464 underwent randomization. The trial was stopped due to a vaccine efficacy of 50% (95% CI 7 to 73). While the primary outcome for this trial was maternal CMV infection, a trial to evaluate protection of symptomatic congenital infection would require the enrollment of more than 50,000 women with an assumed 50% rate of vaccine efficacy.[12] So while vaccine research is very promising and will likely move toward phase 3 trials, there are significant barriers to overcome prior to vaccine prevention of congenital infection.

2.3 Safety

Immune globulin has been used during pregnancy for a number of indications, including hepatitis B exposure, varicella exposure, immune thrombocytopenia and neonatal alloimmune thrombocytopenia. The most common side effect appears to be headaches, which are usually ameliorated by slowing the infusion rate. Few, if any, adverse neonatal effects have been reported. Preparations are manufactured using pooled human plasma from screened donors. The preparations are highly purified polyvalent IgG (>90%) produced by cold alcohol fractionation from the plasma of several thousand volunteers. Preparative techniques of immunoglobulin purification remove relevant transfusion-related viruses (HIV, hepatitis). Contraindications to therapy include known hypersensitivity to immune globulin or IgA deficiency.

Cytomegalovirus immune globulin (CMVIG) is prepared from IgG antibodies derived from pooled healthy, adult donors with high antibody titers to CMV. Cytogam®, from CSL Behring, is the only commercially available CMV specific hyperimmune globulin preparation licensed in the United States. It is FDA approved for use in CMV prophylaxis in transplant recipients who are CMV seronegative and are receiving organs from CMV seropositive donors. These patients are immunosuppressed and at risk for invasive CMV disease. Clinical trials have demonstrated the efficacy of CMVIG in preventing CMV disease in renal transplant recipients.[13]

At usual dosing ranges (50-150 mg/kg), there are few reported side effects from CMVIG infusions. A prospective randomized trial of 99 transplant recipients reported one patient withdrew due to an allergic reaction to the CMVIG. Forty women were excluded after randomization leaving 59 women in the final analysis. Twelve percent of the 59 patients (24 CMVIG, 35 placebo) and 6% of the 205 infusions

analyzed reported a possible side effect and none of the infusions were stopped for any of the reactions noted. The most common side effect reported was flushing.[13] The 24 patients given CMVIG were pooled with an open-label study of 36 patients. Side effects were reported in 18 (30%) patients and 5.2% of the 403 infusions and none of the reactions were severe enough to cause discontinuation of therapy.[14] The most likely CMVIG reactions were chest tightness, muscle cramps, back pain, flushing, and chills. Most of the reactions were rate related and could be eliminated by slowing the infusion rate. A randomized trial of 146 patients reported 17 (23%) in the CMVIG group compared with 8 (11%) in the placebo group (1% albumin) developed a possible reaction (p=0.08).[15] Possible reactions were noted in 6.7 % of the CMVIG infusions and 3.8% of placebo infusions and in only one patient was the effect severe enough to warrant discontinuation of the infusion due to hemolysis.

There are several other human immune globulin products in clinical use and similar cautions are advised with the use of Cytogam® that have been extrapolated from the use of such products. Cytogam® should be used with caution in people with a history of renal dysfunction or volume depletion.[16] A measure of BUN and serum creatinine and adequate volume should be assessed prior to administration.

Other side effects that have been reported with human immune globulin infusion products include anaphylaxis, aseptic meningitis, thrombotic events, hemolysis, and transfusion-related acute lung injury (TRALI). These severe side effects have been noted in case reports of older patients with medical complications and at much higher doses. In the studies discussed above, there were only 2 reports of severe effects that required discontinuation (1 allergic reaction, 1 hemolysis). The incidence of minor side effects such as flushing, chills, muscle cramps, back pain, fever, nausea, vomiting, arthralgia and wheezing were less than 6.0% of all infusions and are most often related to infusion rates.[16] The U.S. FDA category for the commercially available product sold as Cytogam® is pregnancy category C. There were no adverse maternal or fetal events reported in the one study conducted in pregnant women in which 68 women received CMV hyperimmune globulin (31 received 200 U/kg and 37 received 100 U/kg). [4]

2.4 Rationale for a Randomized Clinical Trial

In large part due to the difficulties encountered with serologic screening and diagnosis, as well as the lack of effective preventive strategies following maternal infection, screening for CMV in the U.S. has been limited to those women at high risk due to occupational exposure and/or ultrasound abnormalities. Serologic screening for CMV infection in the first trimester is a common practice in Europe, and recent developments have made CMV screening with subsequent maternal therapy to prevent fetal infection feasible. These include the development of CMV IgG avidity testing, with low avidity antibody suggesting recent infection, and publication of an observational study of the use of CMV hyperimmune globulin to prevent fetal infection following maternal CMV infection (and to treat documented fetal infection).[4]

A trial may establish the feasibility of widespread screening for primary CMV infection during pregnancy, thus changing the practice of prenatal care in the United States. A prospective trial, if the null hypothesis is proved, may also prevent ineffective and unnecessary treatment of pregnant women with an expensive therapy. In summary, due to the large public health, emotional and financial burden of this common disease, improved serologic methods for detecting maternal primary CMV infection, a promising (but unproved) intervention for the interruption of intrauterine transmission, and the current lack of an effective vaccine, a well designed, conducted, and analyzed clinical trial is needed.

2.5 Immune Correlates of Protection

Natural immunity to CMV is highly protective against congenital CMV transmission, as the risk of congenital CMV transmission is reduced from 40% in the absence of preexisting maternal immunity to <2% in mothers with preexisting anti-CMV immunity. Moreover, disease severity in the infant is highly ameliorated in the setting of preexisting maternal immunity. High-avidity maternal antibody responses have previously been demonstrated to modulate the risk of fetal transmission, however the epitope-specificity and functions of protective antibodies have not been established. Moreover, there is limited knowledge of the epitope-specificity of CMV-specific antibodies that can neutralize circulating CMV strains. In addition, it is well-established that CMV-specific cellular immune responses are required for containment of CMV replication, and thus are likely to play a role in protection against congenital CMV transmission. The presence of potent functional anti-CMV antibody responses and strong CMV-specific cellular immune response may correlate with a decreased risk of congenital CMV transmission. Defining protective maternal immune responses in this trial will provide immunologic targets for design and evaluation of candidate maternal CMV vaccines.

3 Study Design

3.1 Primary Research Question

This study will address the primary research question: does maternal administration of CMV hyperimmune globulin lower the rate of congenital CMV infection among the offspring of women who have been diagnosed with primary CMV infection during early pregnancy?

3.2 Secondary Research Questions

Secondary research questions this study will address are:

- Does CMV hyperimmune globulin prevent or lessen the severity of sequelae of CMV infection as assessed at the age of two?
- Does CMV hyperimmune globulin affect neurodevelopmental level as determined by the Bayley Scales of Infant Development III at the age of two?
- Does viral load measured in maternal blood predict adverse neonatal outcome or response to CMV hyperimmune globulin?
- Does maternal humoral and cellular immune response protect against congenital CMV transmission?

3.3 Design Summary

The study is a randomized double-masked placebo controlled multi-center clinical trial of women at participating MFMU Network clinical centers and participating military clinical centers. Women with evidence of primary CMV infection determined from the Abbott Architect system before 23 weeks' gestation will be randomized to one of two monthly treatments:

- IV CMV hyperimmune globulin at a dose of 100mg/kg current body weight
- Identical appearing placebo consisting of IV Albumin 5% diluted in a solution of dextrose in water, (with matching volume). Albumin allows the placebo to have a "foamy" appearance similar to the active drug.

3.4 Eligibility Criteria

3.4.1 Inclusion Criteria

1. Diagnosis of primary maternal CMV infection is defined as one of the following:
   - A positive CMV IgM antibody (≥ 1.00 Index) and low-avidity maternal CMV IgG antibody screen (< 50.0%)
   - Evidence of maternal seroconversion with development of CMV IgG antibody (≥ 6.0 AU/ml) following a prior negative CMV screen (< 6.0 AU/ml)

   The CMV IgG, IgM, and IgG avidity are measured on the Abbott Architect system. The sensitivities and specificities for the three assays range from 97-100%. The IgG avidity which determines a primary infection has a specificity of 100%.[16] The avidity assay has also been shown to correlate much more highly with congenital CMV infection, than using IgM alone and is comparable to seroconversion seen with serial maternal CMV IgG titers. [8]

2. Gestational age at randomization no later than 23^6 weeks based on clinical information and evaluation of the earliest ultrasound as described in "Gestational Age Determination" in Section 3.4.2 below; or
3. Gestational age at randomization no later than 27^6 weeks for the following:
    - Women with a positive IgM and negative IgG, initially screened before 23 weeks who are rescreened after 2 weeks and have evidence of IgG seroconversion
    - Women with initially negative IgM and negative IgG, rescreened after 8 weeks with evidence of seroconversion before 23 weeks
4. Randomization must occur within six weeks of the blood draw for the qualifying screening sample.
5. Singleton pregnancy. A twin pregnancy reduced to singleton (either spontaneously or therapeutically) before 14^0 weeks by project gestational age (see Section 3.4.2 below) is acceptable.

3.4.2 **Gestational Age Determination**

Gestational age is determined using criteria proposed by the American Congress of Obstetricians and Gynecologists, the American Institute of Ultrasound in Medicine and the Society for Maternal-Fetal Medicine and is denoted "project gestational age".[17] The "project EDC", which is based on the project gestational age, cannot be revised once a determination has been made. In the case of in-vitro fertilization, project gestational age is calculated from the date of embryo transfer and the embryo age at transfer.

The following algorithm is used:

1. The first day of the last menstrual period (LMP) is determined, and a judgment made as to whether or not the patient has a "sure" LMP date.
2. If the LMP date is unsure, the ultrasound measurements obtained at the patient's first dating ultrasound examination are used to determine the project gestational age. If the first dating ultrasound was conducted before 14 weeks 0 days, the measurement must be based on crown rump length (CRL).
3. If the LMP date is sure, project gestational age is determined by a comparison between the gestational age by LMP and by the earliest dating ultrasound.
    - If the earliest dating ultrasound confirms the gestational age by LMP within the number of days specified in Table1 below, the LMP-derived gestational age is used to determine the project gestational age.
    - If the ultrasound determined gestational age does not confirm the LMP generated gestational age within the number of days specified in Table 1, the ultrasound is used to determine the project gestational age.

Table 1. Cutoffs for Using LMP to Determine Gestational Age for Sure LMP

Gestational age at first ultrasound by LMP	Ultrasound method of measurement	Ultrasound agreement with LMP
Up to 8 weeks 6 days	CRL	± 5 days
9 weeks 0 days to 13 weeks 6 days	CRL	± 7 days
14 weeks 0 days to 15 weeks 6 days	Per institution	± 7 days
16 weeks 0 days to 21 weeks 6 days	Per institution	± 10 days
22 weeks 0 days to 27 weeks 6 days	Per institution	± 14 days

3.4.3 Exclusion Criteria

1. Maternal CMV infection pre-dating pregnancy as defined by a high IgG avidity index or a positive IgG in the presence of a negative IgM.
2. Known hypersensitivity to plasma or plasma derived products
3. Planned termination of pregnancy
4. Known major fetal anomalies or demise
5. Maternal IgA deficiency, because of the potential for developing antibodies to IgA and the chance of a maternal anaphylactic reaction to subsequent administration of blood products containing IgA, including Cytogam®.
6. Planned use of immune globulin, ganciclovir, or valganciclovir
7. Maternal renal disease (most recent pre-randomization serum creatinine ≥ 1.4 mg/dL; all women must have serum creatinine measured during the pregnancy and prior to randomization), because IV immune globulin products have been reported to be associated with renal dysfunction.
8. Maternal immune impairment (e.g., HIV infection, organ transplant on anti-rejection medications), because this may require use of IV immune globulin products
9. Findings on pre-randomization ultrasound suggestive of established fetal CMV infection (cerebral ventriculomegaly, microcephaly, cerebral or intra-abdominal calcifications, abnormalities of amniotic fluid volume, echogenic bowel or ascities). Abnormally low amniotic fluid volume is defined as no fluid prior to 14 weeks or maximum vertical pocket < 2 cm on or after 14 weeks gestation. Abnormally high amniotic fluid volume is defined as > 10 cm.
10. Positive fetal CMV findings from culture (amniotic fluid) or PCR.
11. Congenital infection with rubella, syphilis, varicella, parvovirus or toxoplasmosis diagnosed by serology and ultrasound or amniotic fluid testing.
12. Intention of the patient or of the managing obstetricians for the delivery to be outside a MFMU Network center, unless protocol subcommittee approval is obtained for that specific delivery location, which will be conditional on special provisions being made to ensure collection of samples to determine the primary outcome, and IRB approval at the delivery hospital.
13. Participation in another interventional study that influences fetal or neonatal morbidity/mortality or developmental outcome
14. Unwilling or unable to commit to 2 year follow-up of the infant

3.5 Informed Consent Criteria

Written informed consent must be obtained from patients before they can be screened for the study by CMV testing (IgM, IgG, and IgG avidity). Patients who are eligible for the study as a result of the screening will be asked to sign another consent form to participate in the trial. Full disclosure of the nature and potential risks of participating in the trial is to be made.

Each center will develop its own consent forms according to the requirements of its own institutional review board using the model consent forms in Appendix B. Each center will also develop its own patient research authorization documents, as required by the HIPAA Privacy Rule, following the guidelines of its own institution. A copy of the signed screening consent form and, if applicable, the consent form for the study will be provided to the patient.

Women who are not fluent in English will be enrolled by a person fluent in their language. Both verbal and written informed consent and authorization will be obtained in that language; if this is not possible the patient will be excluded.

3.6 Randomization Method and Masking

Consenting women will be assigned to one of the two treatment groups by the center's pharmacist, who is not masked, according to a randomization sequence prepared and maintained centrally by the Biostatistical Coordinating Center (BCC). The active and placebo study medication will be prepared at the center's pharmacy according to the randomization sequence. The study is double masked; neither the patient nor the clinical staff will be aware of the treatment assignment.

The simple urn method will be used to generate the randomization sequences because it provides a high probability of balance in treatment assignments, it is unpredictable, and it allows an explicit randomization analysis[18,19] to be conducted with relative ease. Randomization will be stratified by clinical site to assure balance between the two treatment groups with respect to anticipated differences in the clinic populations and possible differences in patient management.

4 Study Procedures

4.1 Screening for Eligibility and Consent

All women presenting for prenatal care before approximately 23 weeks gestational age without a known multifetal gestation are eligible for CMV screening. Prior to testing, if a patient appears to meet the criteria for screening, she will be told about the study and asked to sign an informed consent form for screening (see Appendix B.1 for the model screening consent form). All women who are screened will be provided with the CDC guidelines for prevention of CMV.

Five ml of blood is required for the screening CMV test. The blood preferably will be drawn at the same time that blood is drawn for routine prenatal laboratory testing, and the sample sent to a central laboratory. Both IgM and IgG antibody testing will be employed. If IgM and IgG are positive, the sample will be reflexively assayed for IgG avidity. Validation testing has been performed on all three tests by the central laboratory. The central laboratory for the study will be the Department of Microbiology at the University Health Network and Mount Sinai Hospital in Toronto, Canada.

The results will be forwarded by the laboratory directly to the Biostatistical Coordinating Center (BCC). There are two indications for re-screening patients:

- A positive IgM antibody and negative IgG antibody. The IgG antibody may be repeated in 2 weeks. For these women, randomization must be performed by 27^6 weeks.
- A negative IgM antibody and negative IgG antibody. Screening may be repeated in 8 weeks if the gestational age is still below 23 weeks gestation. For these women, randomization must be conducted by 27^6 weeks.

All positive results will be retested by the laboratory before reporting to the BCC.

The clinical center staff will review the inclusion/exclusion criteria with the medical records of those patients who are identified as having a primary CMV infection to determine whether they are potentially eligible. If the patient is interested after the results are discussed with her, the research nurse will complete the eligibility determination, and inform the patient about the study in detail and request informed consent for the trial. An ultrasound must be performed to evaluate the fetus for signs of established fetal CMV infection. Signs of established infection include: cerebral ventriculomegaly, microcephaly, cerebral or intra-abdominal calcifications, abnormalities of amniotic fluid volume, echogenic bowel or ascities. The ultrasound must be performed after the patient has been screened but before randomization. If the patient has not had a serum creatinine measured during pregnancy, blood will be collected and tested to ensure eligibility. Patients must be randomized within 6 weeks of the qualifying screening sample.

4.2 Randomization and Study Drugs

If eligible and no more than 23^6 weeks (27^6 weeks for all those re-screened with seroconversion), the patient will be randomized. The study nurse will complete the randomization order form which will be sent to the center's pharmacy. The center's pharmacist will use a secure Internet website created by the BCC to determine the next treatment assignment and to randomize the patient before preparing the assigned study drug which is an IV infusion bag.

The study's active drug is Cytogam®, a commercially available product, which is an immunoglobulin G (IgG) containing a standardized amount of antibody to CMV. This drug contains pooled adult human plasma selected for high titers of antibody for CMV, and is administered intravenously at a dose of 100 mg/kg body weight. The 100mg/kg dose of Cytogam® to be used in this trial was chosen based on an approximation to the study performed by Nigro et al.[4] in which a similar European product, Cytotect

Biotest® was used. The maximum recommended dose for Cytogam® is 150 mg/kg, therefore the proposed dose for this study is within the range of that which has been tested for safety. The matching placebo consists of AlbuRx® 5% diluted 1:9 with D5W. AlbuRx® 5% contains pooled adult human plasma. Albumin has been used in a number of studies in pregnancy and has not been associated with any adverse events.[20,21]

The initial infusion of study drug is administered to the patient immediately after it has been received from the pharmacy.

4.3 Baseline Procedures

In addition to information collected for eligibility, project gestational age, and project EDC determination, the following information will be obtained at randomization from a patient interview followed by a review of her chart:

- Demographic information: age, race, insurance status
- Medical history: pre-pregnancy weight, current weight, height, chronic disease history, transfusion history
- Obstetrical history including outcome of all prior pregnancies and history of vaginal bleeding
- Social history: marital status, years of education, alcohol use, tobacco use and other maternal drug use
- Use of day care by children, ages of children living at home
- Current pregnancy complications, including infections.
- Maternal occupation

A 20ml maternal blood sample and a 20ml urine sample will be collected at randomization to assess viral load, humoral and immune response and to conduct future pharmacogenomic studies.

4.4 Study Procedures

Study nurses will stress the importance of clinic attendance and treatment adherence to minimize noncompliance and dropouts. Patients will be kept in the trial even if compliance is less than optimal. If further treatment is refused, follow-up information will continue to be collected. In the event of an unplanned hospitalization, every attempt should be made to have the patient continue the study medication.

Patients will be seen monthly (every 4 weeks) until delivery. At the monthly visits, clinical center staff will assess whether the patient has any side effects, been administered ganciclovir or open-label immune globulin, and will review her medical chart for complications. The study medication will be dispensed by the pharmacy and administered to the patient at each monthly visit. Additional blood (5ml) and urine (5ml) specimens will be collected before each infusion. These samples will be stored to conduct future pharmacogenomic studies and for viral load testing.

4.4.1 **Study Drug Administration**

Infusion rates and monitoring shall be in accord with recommendations on the FDA-approved prescribing information (label) for Cytogam®. All infusions will be administered in a healthcare setting. The infusion will be administered intravenously at 15 mg Ig per kg body weight per hour. If no adverse reactions occur after 30 minutes, the rate may be increased to 30 mg Ig/kg/hr; if no adverse reactions

occur after a subsequent 30 minutes, then increased to a maximum rate of 60 mg Ig/kg/hr (volume not to exceed 75 mL/hour).

The rate of subsequent infusions may be accelerated, per the FDA-approved prescribing information (label) for Cytogam®. The infusions should be administered at 15 mg Ig/kg/hr for 15 minutes. If no adverse reactions occur, the rate may be increased to 30 mg Ig/kg/hr for 15 minutes and then increased to a maximum rate of 60 mg Ig/kg/hr (volume not to exceed 75 mL/hour).

4.4.2 Safety Monitoring

Safety monitoring will be performed before, during and after each study infusion. Nursing or trained medical infusion staff will monitor directly throughout the duration of the infusion in accordance with the infusion policy at the institution. Patients will also be monitored following the infusion by telephone. Table 2 details the minimum safety monitoring that will be performed for each study infusion.

Table 2. Safety Monitoring Schedule for Study Infusions

Monitoring	**Action**
Pre-infusion	Vital signs, check fetal heart rate
Throughout infusion	Medical supervision
Before any rate increase	Vital signs
Midway during the infusion	Vital signs
Post-infusion	Vital signs, check fetal heart rate
16-24 hours after the end of the infusion	Telephone call to patient
Additional monitoring for the first infusion	
8 hours from initiation of the infusion	Medical supervision
OR 4-8 hours after the end of the infusion	Telephone call to patient

In the event that hypotension or anaphylaxis occurs (expect very rarely), study infusions will be discontinued immediately. Hypotension is defined as either a systolic blood pressure < 80 or a diastolic blood pressure < 40 and accompanied by at least a drop of 15 systolic or 10 diastolic.

Anaphylaxis is defined as an acute syndrome occurring within minutes to hours of administration of the study drug and consisting of one or more of the following sets of symptoms:

1. Angioedema (swelling) of the lips, tongue, uvula and/or throat
2. Generalized hives, flushing or pruritus accompanied by clinically significant hypotension (usually with tachycardia)
3. Objective signs of dyspnea such as stridor and/or wheezing

Flushing, generalized pruritis or hives alone would not meet criteria for diagnosis of anaphylaxis. However, if those dermatologic symptoms appear, the patient should be monitoring closely for development of additional signs of anaphylaxis.

For the first infusion, the patient must either stay under medical supervision at the clinical center until 8 hours after the start of the infusion or the study staff must call her 4-8 hours after the end of the infusion. All patients enrolled into the study will be called 16-24 hours after the end of each infusion. On these calls research personnel will ask the patient if she has signs or symptoms of adverse events including numbness, dizziness, confusion, new onset swelling, pain or erythema in one or both legs, changes in urine volume or color. The research staff will also ask the patient if she has had a fever, shaking chills, or rash. If any of these are present additional details will be solicited to determine if it is related to an adverse event or simply due to pregnancy. If the call suggests that an adverse event or complication may be present, the patient will be requested to get medical attention appropriate to the condition. For example if the monitoring reveals that any subject has evidence of possible thromboembolism, an appropriate medical evaluation shall be performed. If a cerebrovascular accident or transient ischemic attack is suspected, the subject will be transferred or referred to the nearest emergency room so that a physical examination and imaging study may be conducted. Therapy will be determined based upon the ensuing diagnosis. If a deep venous thrombosis is suspected, the subject will be referred for physical examination assessment. If the physical examination is concerning for a thromboembolism, imaging studies, such as venous Doppler evaluation, will be performed. If a pulmonary embolus is suspected, the subject will be referred for emergent care including physical examination and radiologic studies as indicated. If there is a high index of clinical suspicion, empiric therapy may be begun while awaiting imaging studies depending upon local care protocols. Imaging studies that may be done include ventilation-perfusion scans, CT-angiography, or angiography. Either low molecular weight heparin or unfractionated heparin are the medications of choice for venous thromboembolism in pregnancy. The research team will inform the clinical care team of any symptoms that may be related to thromboembolism.

4.5 Patient Management and Follow-up

Other than study medications given by protocol, women will receive standard care as defined by their institution/treating physician. An amniocentesis will not be performed for this study and having this procedure will be at the discretion of the patient's physician. Similarly, the only study ultrasound, if not done clinically, will be done prior to enrollment; serial ultrasounds are at the discretion of the patient's physician. In the event of a fetal death (miscarriage, termination, or stillbirth) the products of conception or placenta in the case of stillbirth should be sent for pathologic evaluation for CMV infection. Participation in this study will not preclude the infants from receiving antiviral therapy post-natally.

Pediatricians will be informed at birth that the infant's mother had a primary CMV infection during her pregnancy. Whether or not the infant is quarantined in the nursery is up the individual institution. Study forms detailing pregnancy and neonatal outcome information will be completed prior to mother-infant discharge. Head circumference will be measured on the neonate within 72 hours after birth. If a neonatal complete blood count including liver function tests was not performed, a blood specimen will be collected. Within 2 days of birth and before any treatment, urine and saliva (mouth swab) will be collected on the neonate. Both will be subjected to culture and PCR. If the PCR is positive for either urine or saliva but both cultures are negative, then urine and saliva should be re-collected within 3 weeks of age for confirmatory testing. The neonatal urine and saliva testing will be tested at a central laboratory. A baseline audiologic assessment should be made by a pediatric audiologist within the first month of life. This assessment should include a nonsedated auditory brainstem response (ABR), middle ear immittance measures (tympanometry and acoustic reflex testing) and otoacoustic emissions. Maternal and child contact information will be verified and recorded prior to discharge, along with secondary contact numbers.

The infants born to subjects in this research study will be followed for two years. In order to keep in touch with the infant, follow-up coordinators will use a variety of methods, including contacting the mother by telephone every six months. Visits will be performed at 12 and 24 months. At each visit, a saliva sample on the infant will be collected to assess viral load and at the 24 month visit a saliva sample

will be collected and stored for future DNA research. In addition, the following information will be obtained:

- Postnatal treatment for CMV infection
- Medical history

Developmental testing using the Bayley III and hearing testing will be performed at the 24 month visit. The hearing testing includes visual reinforcement audiometry (VRA), middle ear immittance measures (tympanometry and acoustic reflex testing) and otoacoustic emissions. If hearing loss is suspected, bone conduction tests will also be performed to determine the type of hearing loss. For any child who cannot be reliably conditioned, a referral for further testing by an audiologist or otolaryngologist may be needed. The decision for an audiological referral will be made by site personnel.

Hearing testing will be performed by pediatric audiologists and developmental testing will be performed by examiners that are trained and certified to ensure consistency. Hearing test results will be centrally reviewed by an expert audiologist.

4.6 Adverse Event Reporting

Detailed information concerning adverse events and will be collected and evaluated throughout the conduct of the protocol.

The NICHD Project Scientist and the BCC will be notified within seventy-two hours of any maternal death, neonatal death, or life threatening maternal event by email/phone/fax, if the event occurred in a MFMU Network hospital. For any maternal death, neonatal death, or life threatening maternal event occurring outside a MFMU Network hospital, the adverse event must be reported to the NICHD and the BCC within twenty-four hours of being notified. These and other adverse events deemed serious, unexpected and definitely, possibly or probably related, will be immediately (within twenty-four hours of notification) be forwarded by the BCC to the DSMC Chair, NIH representative, and any other DSMC member who requests notification. If a death is reported, a copy of the patient's medical record will be made. Adverse events which don't qualify under the above definition must be reported to the BCC within 7 days of being notified. These adverse events will be collected and sent to the Chair, NIH representative, and any other requesting DSMC member on a monthly basis. The Chair decides whether the adverse event reports should be disseminated to the rest of the committee, whether a follow-up call or meeting is required, and whether the treatment assignment should be unmasked. NICHD representatives may also request follow-up of specific events. All adverse events will be considered along with other interim safety data in the DSMC deliberations.

An IND safety report will be completed for any suspected adverse reaction that is both serious and unexpected.

The only indication for breaking the randomization code is when it is medically necessary to unmask the study drug assignment to be able to treat the patient.

4.7 Study Outcome Measures and Ascertainment

4.7.1 **Primary Outcome**

The primary outcome is defined as fetal loss (spontaneous or termination), confirmed fetal CMV infection from amniocentesis, neonatal death before assessment of CMV infection can be made, or neonatal congenital CMV infection. Neonatal urine and saliva should be collected by 3 weeks of age (the intent will be to obtain in the first two days of life and before any treatment) for culture and PCR testing. In the event that both the urine and saliva cultures are negative, but the PCR is positive for one or both, urine

and saliva should be re-collected within 3 weeks of age for confirmatory testing. Neonatal congenital CMV infection is diagnosed by urine or saliva that is positive for CMV by culture or repeat PCR.

4.7.2 **Maternal Secondary Outcomes**

1. Gestational hypertension
2. Preeclampsia
3. Placental abruption
4. Gestational age at delivery and preterm birth < 37 weeks' gestation or < 34 weeks' gestation
5. Side effects
6. Treatment emergent serious adverse event. This is defined as any of the following:
    - Maternal death defined as a death of a women while pregnant irrespective of the duration and the site of the pregnancy, from any cause related to or aggravated by the pregnancy or its management but not from accidental or incidental causes
    - Anaphylaxis defined as an acute syndrome occurring within minutes to hours of administration of the study drug and consisting of one or more of the following sets of symptoms:
        - Angioedema (swelling) of the lips, tongue, uvula and/or throat
        - Generalized hives, flushing or pruritus accompanied by clinically significant hypotension (usually with tachycardia)
        - Objective signs of dyspnea such as stridor and/or wheezing
    - Pulmonary embolism confirmed by spiral CT (or strong suspicion on VQ scan if spiral CT not done)
    - Deep vein thrombosis confirmed by venography or Doppler ultrasound, and requiring anticoagulation
    - Stroke as diagnosed by MRI or CT scan and associated with neurologic finding
7. Viral load

4.7.3 **Fetal and Neonatal Secondary Outcomes**

1. Fetal and neonatal mortality
2. Primary outcome excluding terminations
3. Head circumference measured within 72 hours of birth
4. Birth weight
5. Growth restriction defined as $<5^{th}$ percentile weight for gestational age, assessed specifically by sex and race of the infant based on United States birth certificate data[22]
6. Microcephaly defined as $<3^{rd}$ percentile head circumference for gestational age, assessed specifically by sex of the infant based on Olsen's data from a U.S. population[23]
7. Symptomatic CMV infection defined as CMV isolated from an amniocentesis, or urine or saliva during the first three weeks of life and at least one of the following: jaundice (direct bilirubin exceeding 20% of the total bilirubin), hepatomegaly, splenomegaly, growth restriction, intracerebral calcifications, microcephaly, hypotonia, seizures, petechial rash, hearing loss,

interstitial pneumonitis, thrombocytopenia (defined as platelet count <100,000/mm^3), anemia (defined as a hematocrit of <35%), hepatitis (defined as AST or ALT ≥100 U/L), chorioretinitis, or CMV in cerebrospinal fluid.

8. Intraventricular hemorrhage (IVH) grades III or IV as determined by cranial ultrasounds performed as part of routine clinical care and classified based on the Papile classification system
9. Ventriculomegaly
10. Retinopathy of prematurity (ROP). This diagnosis will be reached when an ophthalmologic examination of the retina has been performed and ROP is diagnosed at Stage I (demarcation line in the retina) or greater.
11. Respiratory distress syndrome (RDS) defined as the presence of clinical signs of respiratory distress (tachypnea, retractions, flaring, grunting, or cyanosis), with an oxygen requirement and a chest x-ray that shows hypoaeration and reticulogranular infiltrates.
12. Chronic lung disease (BPD) defined as oxygen requirement at 28 days of life.
13. NEC, defined as modified Bell Stage 2 or 3. Stage 2: Clinical signs and symptoms with pneumatosis intestinalis on radiographs. Stage 3: Advanced clinical signs and symptoms, pneumatosis, impending or proven intestinal perforation.
14. Hyperbilirubinemia. Peak total bilirubin of at least 15 mg% or the use of phototherapy
15. Neonatal infectious morbidity
    - Sepsis (within 72 hours and > 72 hours after birth). The diagnosis of sepsis will require the presence of a clinically ill infant in whom systemic infection is suspected with a positive blood, CSF, or catheterized/suprapubic urine culture; or, in the absence of positive cultures, clinical evidence of cardiovascular collapse or an unequivocal X-ray confirming infection.
    - Suspected sepsis. The diagnosis of suspected sepsis will include infants with suspicious clinical findings of infection, but no positive cultures or X-rays.
    - Pneumonia. The diagnosis of pneumonia will be confirmed by X-ray or positive blood culture.
16. Seizures / encephalopathy
17. Length of hospital stay, need for NICU or intermediate care admission and length of stay if admitted

4.7.4 Infant and Child Secondary Outcomes

1. Infant or child death
2. Sensorineural hearing loss (unilateral and bilateral) at 24 months
3. Chorioretinitis defined by ophthalmologic exam
4. Cognitive and Motor Scores from the Bayley Certified Scales of Infant Development III at 24 months corrected age
5. Composite outcome at 24 months including any of the following attributable to congenital CMV infection:
    - Sensorineural hearing loss (unilateral and bilateral)

- Developmental delay defined as Cognitive score < 70 or Motor score < 70 on the Bayley III
- Chorioretinitis
- Seizure disorder as a result of CMV infection
- Fetal loss or death of neonate, infant or child

6. Child status at age 24 months, classified as:
    - Fetal loss or death of neonate, infant or child
    - Congenital CMV infection with severe disability
    - Congenital CMV infection without severe disability
    - Infant not infected with CMV
7. Failure to thrive defined as $<10^{th}$ percentile for weight at 12 and 24 month
8. Viral load

5 Statistical Considerations

5.1 Data Relevant to the Primary Outcome

A large meta-analysis suggests that 32% of infants will be infected after a primary maternal infection.[24] This is a conservative estimate because the study by Nigro et al found a 40% congenital infection rate in the placebo group.[4] The study by Nigro et al reported a 60% reduction in primary congenital CMV infection in the women treated with CMV hyperimmune globulin.

5.2 Sample Size and Power

The primary outcome for this study is fetal loss or neonatal congenital CMV infection. The termination and fetal loss rate in the placebo group is expected to be 10% and of the remaining 90%, the neonatal congenital infection rate will be 32%. Therefore, the overall primary outcome rate in the placebo group is expected to be 38%. Assuming a lower termination and fetal loss rate of 7% in the treated group and a 30% reduction in the infection rate, a total sample size of 800 subjects, or 400 per group, is sufficient to detect the difference of 38% to 26.6% with power of at least 90 percent and type-I error (2-sided) of 5%.

Table 3. Sample Sizes per Group for Different Primary Outcome Rates, Power and Effect Sizes

		Primary Outcome Rate in Placebo Group		
% Reduction in Infection Rate	% Power	36%	38%	40%
25	80	400	370	350
	85	450	420	390
	90	530	490	450
30	80	310	290	270
	85	350	320	300
	90	400	370	350

The major secondary outcome is an ordinal variable that categorizes the severity of CMV infection into four categories, 1) fetal loss or death of neonate, infant or child, 2) congenital CMV infection with severe disability defined as sensorineural hearing loss, developmental delay (Cognitive score < 70 or Motor score < 70 on the Bayley III), chorioretinitis or seizure disorder, 3) congenital CMV infection without severe disability or 4) infant not infected with CMV. The rates of the four categories will be 11.7%, 5.5%, 21.6% and 61.2% assuming the 10% termination/fetal loss rate and 32% congenital infection rate stated earlier and further assuming that of the 32% of infected infants, 6% will die and 19% will have a severe disability based on data from Fowler, et al.[25] Assuming a 5% lost to follow-up rate and test for trend using a linear rank score, a sample size of 760 subjects is sufficient to detect at least a 30% reduction in each of the three adverse categories (11.7% to 7.8%, 5.5% to 3.7%, and 21.6% to 14.4%) with a power greater than 90 percent and type-I error (2-sided) of 5%.

5.3 Feasibility

The feasibility of answering the primary research question will be addressed after the first 10,000 women are screened. The feasibility of this trial is largely based on the assumptions of a 1% screen positive and

50% consent rate which means 200 women will need to be screened for every woman randomized. An upper limit of 300 women screened for every randomization has been established. If the number is below this limit, feasibility will be assured and the trial will continue as planned. If the upper limit is exceeded, the Data and Safety Monitoring Committee will be consulted for consideration of stopping recruitment.

5.4 Interim Analysis

Interim statistical analyses of clinical trials are a requirement of all National Institutes of Health (NIH) sponsored clinical trials. [26] The Data and Safety Monitoring Committee (DSMC) meets in person at least once per year. For this trial, the DSMC will conduct a six-monthly regular review of trial performance and safety issues. The first full review of safety and performance issues will occur when 50 patients have been randomized and undergone their first infusion. Once 50 women have been randomized, recruitment will be halted until a full review has been conducted by the DSMC, and approval has been received to continue. Subsequent reviews will take place at the time of the annual meeting and at the six month point in between the annual meetings. This timing may be modified by the DSMC.

Before each of the six monthly meetings, a formal detailed report will be written by the Biostatistical Coordinating Center (BCC) which presents all baseline variables, protocol adherence, side effects, all adverse events reported, as well as center performance in terms of recruitment, data quality, loss to follow-up and protocol violations.

Once sufficient patients have been accrued into the trial, the report will also include a formal interim analysis evaluating the primary outcome by treatment group. For this evaluation, a cohort of patients is chosen consisting of all patients randomized before a certain date so that the analysis cohort does not depend on gestational age at delivery. Because women may be randomized early in gestation, this cohort will consist of women randomized at least 9 months earlier. For the first interim analysis of the primary outcome which is anticipated to take place 18 months after study start, it is expected that 150 women (19% of the final sample size) will be in the analysis cohort. The actual number of women in the cohort will depend on the rate of recruitment. This first formal interim analysis will occur at between 15% and 30% of the final sample size (i.e. 120 to 240 women). Subsequent interim analyses of the primary outcome will occur annually or more frequently at the discretion of the DSMC.

The main statistical issue relevant to interim analysis is the problem of performing multiple tests of significance on accumulating data. A number of procedures have been developed to handle this situation.[27,28] Most techniques [29,30] entail adjusting the nominal significance level at the interim evaluations to some value less than α, such that the overall probability of committing a type-I error is maintained at α. For this trial, the group sequential method of Lan and DeMets[31] will be used to characterize the rate at which the type I error is spent. This method is flexible with regard to the timing of the interim analyses. Asymmetric stopping boundaries will be used for the Lan-deMets procedure. The upper boundary which describes the stopping rule for benefit will be based on 1-sided type I error of .025 and the Lan-deMets generalization of the O'Brien-Fleming boundary.

Sometimes, a surprising development may lead to extra looks or even continuous monitoring of the data, such as on a monthly basis. The Lan-DeMets procedure is flexible, in that one can switch from occasional to continuous monitoring of the data, with negligible effect on the type I error level.[32] In some instances the Lan-DeMets boundary for statistical significance may be crossed, but due to other considerations the DSMC decides that the trial should continue. In this case, the type I error previously spent under by the α spending function can be retrieved to be distributed over future looks without inflating the total type I error probability.[33] The lower boundary will be based on a less stringent stopping rule: 1-sided type 1 error of .05 and the Lan-deMets generalization of the Pocock type boundary.

If recruitment proceeds as planned and annual analyses are conducted, it is expected that three interim analyses will be conducted at 19%, 44%, and 69% of the final sample size of 800. This would require critical values of 4.0, 3.19, and 2.47 to cross the boundary for benefit, corresponding to p-values of

.00003, .0007, and .007, respectively. To cross the boundary for harm would require critical values of -2.19, -2.09 and -2.06, corresponding to p-values of .014, .018, and .039. However, this guideline will not preclude the DSMC recommending termination earlier for evidence of harm in the primary outcome.

In addition, the BCC will continuously monitor potential cases of treatment emergent serious adverse events in the active arm during the trial (defined as maternal death, thromboembolic event or anaphlyaxis in Maternal Secondary Outcomes Section 4.7.2). If the number of such events that occur within 72 hours of infusion meets the number in the table below, recruitment for the study will immediately halt until the DSMC has met and reviewed all of the data to determine whether the trial should continue.

Number in Patients in Cytogam® Group	Number of Events
Up to 71	2
72 to 164	3
165 to 274	4
275 to 395	5
396 or more	6

The numbers were calculated assuming a 0.5% rate of one of these events occurring in a pregnancy in the general population, and finding the smallest number of events such that the probability that the observed event rate exceeds 0.5% is less than 5 percent.

The DSMC will also be consulted at any time if safety concerns arise during the conduct of the study. The DSMC receives all adverse event reports on an ongoing basis and may request a review at any time.

The DSMC review will evaluate events occurring in the placebo group as well as the active arm, and will determine whether diagnoses are confirmed as defined above and plausibly temporally related to an infusion.

It is often useful to calculate conditional power given the observed data to date, and conditional on the future data showing the originally assumed design effect.[34] If this conditional power is low (under 10 percent) the DSMC may consider termination for futility if the accrual rate is slow with confidence that the Type II error is not greatly inflated.

It is recognized that any decision to terminate the study would not be reached solely on statistical grounds but on a number of complex clinical and statistical considerations. [35] For this study, it is recommended that the study not be stopped early for benefit unless the benefit is also seen in the secondary ordinal outcome of CMV infection severity.

5.5 Analysis Plan

All statistical analyses will be based upon the total cohort of patients randomized into the trial. Although data on some patients may be missing, all relevant data available from each patient will be employed in the analyses. Patients will be included in the treatment group to which they were randomly assigned regardless of compliance.

The primary analysis will consist of a simple comparison of binomial proportions. The relative risk and confidence interval will be reported. The individual components of the composite outcome will also be examined. If the treatment groups are found to differ on a pre-treatment factor known to be a risk factor for the outcome, the statistical analysis will adjust for these differences. An evaluation of treatment by

center interaction will be included. An analysis adjusting by center also will be performed to ensure that center differences do not change the conclusion.

If the two groups show a difference in the incidence of the primary outcome, interactions will be evaluated and subgroup analyses conducted to determine whether the effect prevails throughout particular subgroups of patients. Indeed, NIH guidelines require investigators to evaluate consistency between the genders and across racial subgroups (see Section 5.5.1). It should be noted, however, that subgroup analyses have been greatly abused,[36] particularly when there is no overall treatment difference. There is a strong temptation to search for a specific subpopulation in which the therapy is nevertheless effective. Yusuf et al[37] concluded "*the overall 'average' result of a randomized clinical trial is usually a more reliable estimate of the treatment effect in the various subgroups examined than are the observed effects in individual subgroups*." Thus subgroup analyses will be interpreted with care.

It is generally acknowledged that subgroup analysis that is pre-specified in the protocol has more validity than ad-hoc comparisons. The following factors will be considered for subgroup analysis, if there is a significant interaction between the factor of interest and the treatment effect.

- Race/ethnicity (see below)
- Gestational age at randomization (<16 weeks and ≥ 16 weeks)
- Viral load at randomization (detectable and not detectable)
- Avidity (< 30% and ≥ 30%)
- Maternal age (< 25 years and ≥ 25 years)
- BMI at randomization (< 30 and ≥ 30)

Loss to follow-up will be defined as the inability to ascertain whether the infant was born alive and if born alive whether the neonate was infected with CMV. Those defined as lost to follow-up will not be included in the primary analysis. It is expected that the loss to follow-up rate will be very low. For trials conducted by the MFMU Network, the loss to follow-up rate has typically been under 2 percent. However, a sensitivity analysis will be performed including patients lost to follow up with different assumptions regarding their outcome, to determine whether the results are robust.

Since many of the secondary endpoints are dichotomous variables like the primary outcome, standard statistical methods for rates and proportions will be appropriate. The Wilcoxon rank sum test will be used to compare continuous variables, and survival analysis methodology may be used to compare time-to-event variables.

In general, analyses of data will be conducted to address the primary and secondary research questions of the trial, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study.

5.5.1 **Racial/Ethnic Subgroup Analysis**

The racial/ethnic composition of patients of women recruited into the MFMU Network trials varies. Assuming for this trial that the composition is 25% African-American and 25% Hispanic, there is a minimum of 80% power to detect a 50% reduction in the primary outcome in each of the two subgroups.

6 Data Collection

6.1 Data Collection Forms

Data will be collected on standardized forms on which nearly all responses have been pre-coded. Each form is briefly described below:

- CM01 Screening Log.
- CM02 Eligibility and Randomization Form is completed for all patients eligible for the study.
- CM03 Randomization Order Form
- CM03A Infusion Order Form
- CM03B Pharmacy Log lists all randomized patients and provides the study drug code numbers. To be completed by the investigational pharmacy
- CM04 Baseline Form is completed for all randomized patients. This form includes detailed demographic and social data, exposure, medical and obstetrical history, and current pregnancy complications.
- CM04A Previous Pregnancy Outcome Form
- CM04B Baseline Ultrasound Form
- CM05 Study Visit Form
- CM05A Side Effect Log
- CM05B Infusion Log
- CM08 Labor and Delivery Summary Form documents specific pregnancy complications since randomization, labor, delivery and postpartum information.
- CM09 Neonatal Baseline Form records date and time of birth, delivery data and status at delivery, for each fetus/infant.
- CM10 Neonatal Outcome Form records outcome data for all infants admitted to the NICU or special care nursery.
- CM11 Patient Status Form documents loss to follow up/withdrawal status, last date of contact for lost to follow-up patients, side effects since the last dose.
- CM12 Adverse Event Form records serious and non-serious adverse events.
- CM13 Follow-up Visit Form
- CM13A Hearing Form
- CM13B Bayley III Form

6.2 Web Data Entry System

For this protocol, web data entry screens corresponding to the study forms listed above will be developed and maintained by the staff of the BCC. Clinical center staff will enter data into the MySQL database located at the BCC through a web data management system (MIDAS). The data are edited on-line for missing, out of range and inconsistent values. A Users' Manual documenting this system is provided to the centers by the BCC.

6.3 Centralized Data Management System

Daily data conversions from the MySQL database create up-to-date SAS datasets. Data are reviewed weekly using edit routines similar to those implemented on-line during data entry, as well as additional checks for data consistency within or across forms. A database of resulting potential data problems is generated in MIDAS for initial review by BCC staff, who then evaluate the comments keyed in association with edits on missing or unusual values. Valid edits will be flagged in MIDAS for resolution at the clinical centers.

At regular intervals, specialized data reviews comparing data availability and consistency across forms are run by the BCC staff on the entire database or on a specific subset of data. These reports are also submitted to the centers for correction or clarification.

An audit trail, consisting of all prior versions of each data form as entered in the computer for each patient, is maintained so that the succession of corrections can be monitored.

6.4 Performance Monitoring

The BCC will present regular reports to the CMV Subcommittee, the Steering Committee, and the Data and Safety Monitoring Committee. These include:

- Monthly Recruitment Reports - reports of the number of women screened and enrolled by month and by clinical center are provided monthly to the CMV Subcommittee and all other members of the Steering Committee. Bi-weekly reports are provided electronically if needed.
- Quarterly Steering Committee Reports - reports detailing recruitment, baseline patient characteristics, data quality, incidence of missing data and adherence to study protocol by clinical center, are provided quarterly to the CMV Subcommittee and all other members of the Steering Committee.
- Data and Safety Monitoring Committee Reports - for every meeting of the DSMC, a report is prepared which includes patient recruitment, baseline patient characteristics, center performance information with respect to data quality, timeliness of data submission and protocol adherence (in addition to safety and efficacy data). The reports also include adverse events, loss to follow-up and all outcome variables as described previously in this protocol.

7 Study Administration

7.1 Organization and Funding

The study is funded by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). The study is conducted by the NICHD Maternal-Fetal Medicine Units (MFMU) Network, selected military clinical centers, the Biostatistical Coordinating Center (BCC) and the NICHD, and is administered under cooperative agreements between each of the centers and the NICHD. Each of the funded institutions is represented by a Principal Investigator.

7.1.1 **Participating MFMU Network Clinical Centers**

The participating Principal Investigators of the MFMU Network clinical centers have agreed to abide by the study protocol, to have comparable staff, facilities and equipment and to ensure the proper conduct of the study at each of their centers including: recruitment and treatment of patients as specified in the protocol, accurate data collection and the transmission of information to the Steering Committee. A complete description of the organization of the MFMU Network is provided in the MFMU Network Policy Manual.

7.1.2 **Participating Military Clinical Centers**

As part of a collaborative effort with the MFMU Network, selected military clinical centers will recruit women for the CMV study. Similar to the MFMU Network, all participating centers will have agreed to abide by the study protocol, to have comparable staff, facilities and equipment and to ensure the proper conduct of the study.

7.1.3 **Biostatistical Coordinating Center**

The BCC is responsible for all aspects of biostatistical design, data management, interim and final statistical analyses, and preparation of publications based on the study results. The Principal Investigator of the BCC reports to the Steering Committee and the Data and Safety Monitoring Committee.

7.1.4 **NICHD**

In addition to its role as funding agency, the NICHD participates in the activities of the Network, including the development of protocols, administration and conduct of the studies and preparation of publications.

7.1.5 **CSL Behring**

CSL Behring is a company located in King of Prussia, Pennsylvania that manufactures the cytomegalovirus immune globulin intravenous treatment. The company has agreed to donate and ship the study drug to the clinical centers. CSL Behring will not have input into design, implementation, data management, or publication of results.

7.1.6 **Network Advisory Board**

Appointed by the NICHD, the members of the Network Advisory Board consist of a group of experts who are not affiliated with research being conducted by the Network and represent the disciplines of maternal-fetal medicine, neonatology and biostatistics/epidemiology. The role of the board includes the review and prioritization of proposed studies, in addition to the identification of scientifically and clinically important questions and ideas that might be conducted by the Network. The NICHD Project Scientist convenes and attends the meetings.

7.2 Committees

7.2.1 Steering Committee

The Steering Committee consists of the Principal Investigator from each of the twelve MFMU Network clinical centers, the principal investigator from the BCC, and the NICHD MFMU Network Project Scientist. They are all voting members and the Chair of the Steering Committee may vote to break a tie. The Chair, a person independent of the participating institutions, is appointed by NICHD. The Steering Committee has the responsibility for identifying topics for Network studies, designing and conducting study protocols and monitoring study implementation, recruitment and protocol adherence. The committee receives recommendations from the Data and Safety Monitoring Committee and the Network Advisory Board.

7.2.2 Protocol Subcommittee

The subcommittee consists of a chair (who is an investigator from one of the clinical centers), investigators from one or more other clinical centers, BCC staff, nurse coordinators, outside consultants (if appropriate), and the NICHD MFMU Network Project Scientist. The Protocol Subcommittee is responsible for the preparation and conduct of the study, and reporting the progress of the study to the Steering Committee.

7.2.3 Publications Committee

The Publications Committee is a standing committee of the Steering Committee. The functions of this committee are to develop publication policies and to review all manuscripts and abstracts prior to submission. The goals of this committee are fair and appropriate authorship credit and high quality publications.

7.2.4 Data and Safety Monitoring Committee

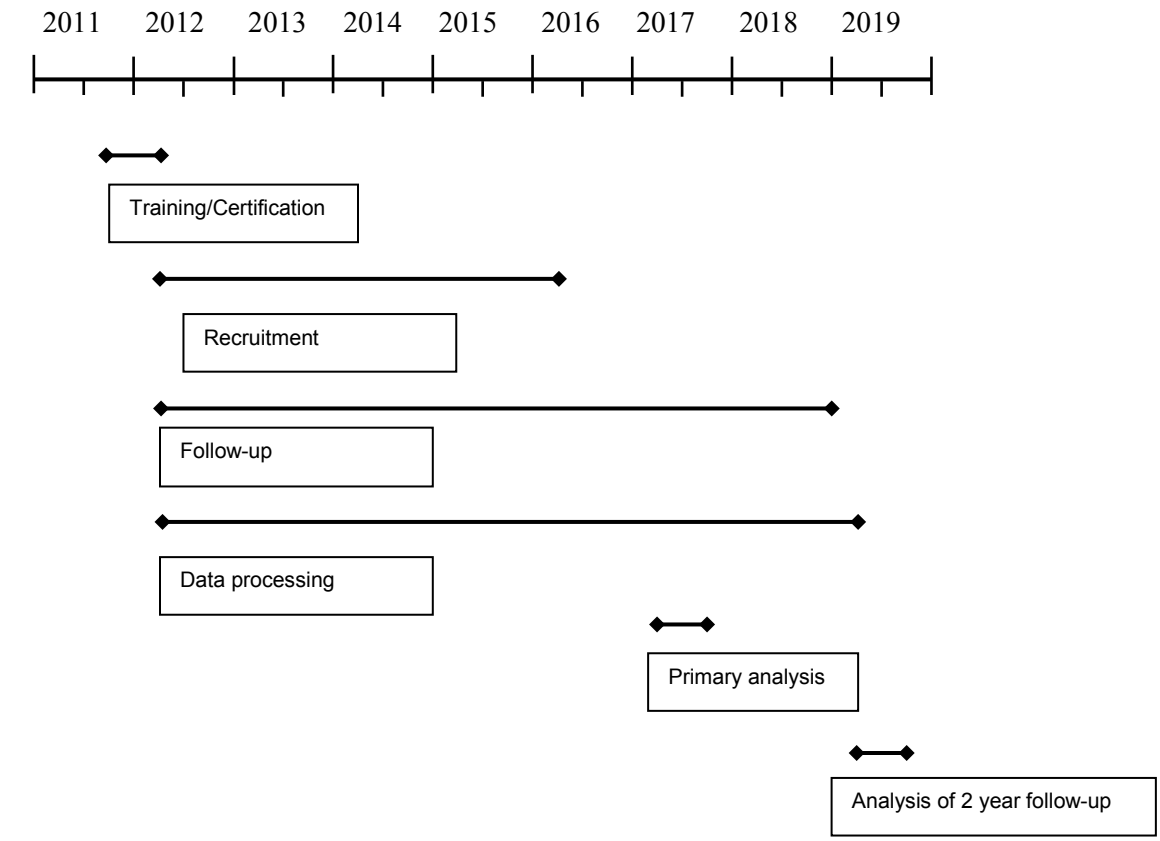
The Data and Safety Monitoring Committee (DSMC), a group of individuals not affiliated with any of the participating institutions, was established by the NICHD. Before the trial can begin, the protocol must be approved by the committee. During the conduct of the study, the committee is charged with monitoring the emerging results for efficacy and safety, in addition to center performance and protocol adherence. Recommendations by the committee can include protocol modification, early termination for efficacy, or for unexpected safety problems. Recommendations are made to the NICHD and disseminated to the Steering Committee.

8 Study Timetable

8.1 Training and Certification

The study timetable as originally planned is depicted below.

Figure 1. Timetable



Once the study start date is set, one month start-up time is allotted to complete final training (and certification), in addition to distribution of study drugs. It is assumed that all other preparation for the trial will have been completed, including obtaining the IND and IRB approval, preparation of the randomization sequence, preparation of the study drugs, implementation of the data entry and management system.

Each participating center must be certified to start the trial before recruitment at that center can begin. The certification requirements are designed to ensure that personnel involved in the trial are committed to the study and proficient in study procedures, and that the center has satisfied regulatory requirements. Each center is required to obtain IRB approval for the study before they are certified to begin the trial.

8.2 Recruitment and Data Collection Period

Assuming a 1% primary maternal infection rate before 23 weeks, and a conservative 50% consent rate, approximately 160,000 women would need to be screened to enroll 800 subjects. Approximately 3,500 women per month underwent serological screening at the peak recruitment for the MFMU TSH trial. At this rate, screening could be accomplished in 4 years.

8.3 Final Analysis

After a two-month period for completion of data entry for the trial and close-out of the delivery and primary outcome, the data set will be locked and available for the primary outcome analysis. Approximately 6 months will be required to submit the study's primary report for publication. No other outcome reports will be presented or published until completion of the infant follow-up. After completion of the 2 year infant follow-up, a two-month period will be dedicated to complete data entry and close-out of this follow-up. Approximately 6 months will be required to complete the final report to the Steering Committee and to submit the study's report on follow-up for publication.

Appendix A Design Summary

A Randomized Trial to Prevent Congenital Cytomegalovirus Infection (CMV)

OBJECTIVE: To determine whether maternal administration of CMV hyperimmune globulin prior to 24^0 weeks gestation in women diagnosed with primary CMV infection reduces the rate of congenital CMV infection.

ORGANIZATION

Clinical Centers:	Magee, UAB, Ohio State, UTSW, Utah, Brown, Columbia, Case Western, UT-Houston, UNC, Northwestern, UTMB-Galveston, Colorado, Duke, Stanford, U Penn, Madigan
Subcommittee:	Brenna Hughes, MD (Chair)

DESIGN

Major Eligibility Criteria:	❖ Singleton gestation ❖ Gestational age $<24^0$ wks ❖ Primary CMV infection
Groups:	❖ CMV hyperimmune globulin (Cytogam®) ❖ Matching placebo (AlbuRx® 5% diluted 1:9 with D5W)
Random Allocation:	Standard urn design; 1:1 allocation
Stratification:	❖ Clinical site
Sample Size:	❖ 800
Assumptions:	❖ Outcome event = fetal loss or neonatal congenital CMV infection ❖ Placebo group event rate = 38% ❖ Experimental group event rate = 26.6% (30% reduction) ❖ Type 1 error = 5% (2-sided) ❖ Power = 90%
Interim Analysis:	Lan-Demets group sequential method

SCHEDULED EVALUATIONS / DATA COLLECTION

Pre-Randomization:	❖ Blood for IgG, IgM and IgG avidity (5ml) ❖ Ultrasound to assess fetal CMV infection ❖ Blood for serum creatinine (2.5 ml)
Randomization:	❖ Pregnancy, exposure and medical history ❖ Blood (20ml) and urine (20ml) for storage
Post-randomization:	❖ Every four weeks: infusion of study medication, assess side effects and open-label use ❖ Blood (5ml) and urine (5ml) for storage
Post-infusion:	❖ Patient stays on site for 8 hrs after start of 1st infusion, or phone call to patient at 4-8 hours after the end of 1st infusion ❖ Phone call to patient at 16-24 hours after the end of all infusions
Delivery:	❖ Delivery and neonatal data
Follow-up:	❖ Urine and saliva on infants by 3 weeks ❖ Hearing by one month ❖ Saliva collection on infants at years 1 and 2; Developmental and hearing testing and saliva for storage at year 2 only

MANAGEMENT PROTOCOL

Coded Medication:	❖ Monthly infusion (CMV hyperimmune globulin dose : 100 mg/kg or placebo) from randomization to delivery

OUTCOME MEASURES

Primary:	❖ Fetal loss or neonatal congenital CMV infection diagnosed by urine or saliva by 3 weeks of age.
Secondary:	❖ Severity of CMV infection ❖ Preterm birth ❖ Fetal loss or death of neonatal, infant or child ❖ Symptomatic CMV infection ❖ Sensorineural hearing loss ❖ Neurologic impairment ❖ Developmental delay

TIMETABLE
(as originally planned)

Enrollment	March 2012 – March 2016
Data Collection	March 2012 – December 2018
Closeout/Final analysis	January 2019 – July 2019

Appendix B Sample Informed Consent Documents

B.1. Sample Informed Consent Form for Screening

Research Study Title: A Randomized Trial to Prevent Congenital Cytomegalovirus Infection (CMV)

Sponsor: *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) of the National Institutes of Health (NIH)

Principal Investigator: _____***Phone*** **(____) ___ - ____**

Introduction

You are invited to be screened to see if you are eligible to take part in a research study. This consent form provides the information about the risks and benefits of the screening portion of this research study. You can choose whether or not you will take part in the study. If you agree to take part, you will need to sign this consent form. This process is known as informed consent.

Research Purpose

Cytomegalovirus (CMV) is a common virus that is spread like a cold or flu. A very small number of pregnant women, approximately 1%, will get infected with CMV for the first time while they are pregnant. If a woman gets infected for the first time during pregnancy, there is a 40% chance that the fetus will also get infected. Fetuses that get infected with CMV are more likely to be born earlier than expected (i.e., preterm), have low birth weight or small heads. A small number of the infected babies will develop problems such as hearing loss, difficulty learning or rarely, the baby may die.

The way we will know whether you have been infected during pregnancy is by testing your blood. The purpose of this study is to see if giving CMV antibodies (i.e. substances that target and help the immune system destroy the CMV) to pregnant women who have been infected with CMV for the first time during pregnancy will result in fewer fetuses being infected with CMV. Since the number of pregnant women infected for the first time is expected to be small, we anticipate that approximately 160,000 women will be screened for this study.

Procedures

If you consent to screening for this study, about a teaspoon of blood will be drawn for CMV antibody tests at the time of your routine prenatal labs, if possible, and sent to a centralized laboratory for testing. Your weight may also be collected. A research nurse may ask you to come back in a few weeks to repeat the blood test to confirm the initial findings.

If you are found to be eligible, you may be approached by a research nurse to consent to the research study which is a randomized, placebo controlled study of treatment with CMV antibodies during pregnancy.

Possible Risks

You may experience the discomforts associated with drawing blood, which may include bruising, discomfort or pain at the site, infection, or fainting.

Benefits

This screening may not benefit you directly. However, if the screening shows that you have been infected with CMV while you are pregnant, you may be eligible for the randomized treatment trial.

Consent for Use/Disposal of Blood

By signing this consent form, you agree to the use of your blood to see if you have been infected with CMV for the first time in your current pregnancy. If any blood remains after testing, you agree that it will be thrown away after 6 months.

Alternatives

The alternative to this study is not to participate.

Costs

There will be no additional cost to you for participation in the research study.

Payment for Injury or Harm

In the event of injury from drawing blood, treatment will be provided.

Right to Withdraw From the Research Study

You are free to withdraw your consent and stop participating at any time.

Confidentiality

You have the right to privacy. All information obtained from this research that can identify you will remain confidential within the limits of the law. The results of your CMV test will be sent to the data coordinating center, The George Washington University Biostatistics Center in Rockville, Maryland, with a unique code. Only the research study staff at this medical center for this study will have access to the key to the code that can identify you.

Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality from the NIH. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless you have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings); if you have consented to the disclosure or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the NIH or to meet the requirements of the Food and Drug Administration (FDA). If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

Questions

If you have questions about the procedures of this research study, please contact _____ by telephoning (___) ___-____ during the workday.

If you have any questions about the informed consent process or any other rights as a research subject, please contact _____, Director of the Office of Human Research, at (____) ___-____. _____ who is your representative.

Signatures

By signing this form you show that you have read this informed consent form, the study has been explained to you, your questions have been answered, and you agree to take part in the screening for this study.

_____	_____	_____
Participant (*Print Name*)	Signature	Date
_____	_____	_____
Person Obtaining Consent *(Print Name)*	Signature	Date

ASSENT FOR FEMALES UNDER 18 YEARS of AGE (if required by Center IRB):

I agree _____ I do not agree_____ to participate in this study.

This has been explained to me by _____

_____	_____
Signature of Minor	Date
_____	_____
Print Name of Subject	Age

Please provide either one or both parental signatures as instructed by your IRB.

_____	_____
Signature of Mother/Guardian	Date
_____	_____
Signature of Father/Guardian	Date

A witness unrelated to the study is necessary if the participant can comprehend but cannot read (i.e., blind), or cannot sign (e.g., unable to use hands) the consent form.

_____	_____	_____
Witness' Name	Signature	Date
(Print Name)		

B.2. Sample Informed Consent Form for RCT

Research Study Title: A Randomized Trial to Prevent Congenital Cytomegalovirus Infection (CMV)

Sponsor: *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) of the National Institutes of Health (NIH)

Principal Investigator: _____*Phone* **(**____) ___ - ____

Introduction

The laboratory test of your blood, which was done to screen you for this research study, suggests that you have been infected with cytomegalovirus (CMV) for the first time (i.e. a primary infection). In this study, you may be given CMV antibodies that might help your immune system destroy the CMV infection so you cannot pass it to your baby. Antibodies are substances that are purified from human blood. Antibodies target and help the immune system to destroy harmful organisms.

Before you decide to be a part of this study, you need to understand the risks and benefits. This consent form provides information about the research study. A staff member of the research study will be available to answer your questions. You can choose whether or not you will take part in the study. If you agree to take part, you need to sign this consent form. This process is known as informed consent.

Research Purpose

CMV is a common virus that usually has few symptoms. When first infected, some people may feel tired, weak, have a fever, or swollen glands. Most people in the United States are infected during childhood or as adults if they work around children. When women get infected with CMV for the first time while pregnant, the virus may infect their baby. About a third of infected babies may develop permanent disabilities including hearing loss and learning difficulties. A small number will die from the infection.

It is not usual practice to screen pregnant women for CMV infection. Also, there is no agreement about how to evaluate and manage pregnant women infected with CMV for first time like you. At this point, there is not enough evidence that any treatment helps the baby.

This research study was set up to find out whether treating pregnant women who have a primary CMV infection with CMV antibodies will lower the number of babies infected with CMV. The research study is funded by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). Medical centers across the country are part of this research study and, in all, 800 pregnant women who are identified with a primary or first-time CMV infection will be enrolled. The children of these women will be tested and evaluated at one and two years of age.

Procedures

If you are eligible and decide to participate in this study, you will have an ultrasound to see if the baby is infected with CMV. We may take a small amount (half teaspoon) of blood to measure how your kidneys are doing. If you are still eligible, you will be randomly put into one of two treatment groups like flipping a coin. One group will receive the active research medication (CMV antibodies) and the other group will receive the placebo, a medication that looks the same, which is water with sugar and protein. Both medications are given through an intravenous (IV) tube.

You, your doctor, and the research study nurse will not know which treatment group you are in or if you are getting the CMV antibodies or placebo. This is to make sure everyone is treated the same. In case of

an emergency, the doctor may call the research pharmacy to find out which group you are in. At the end of the research study, after all of the data have been analyzed, your study nurse may be able to tell you whether you were given CMV antibodies or placebo if you want to know.

Before the first study IV we will collect a small amount (1½ tablespoons) of blood and a small amount (1½ tablespoon) of urine that gets stored. If you agree, a small amount (½ teaspoon) of your blood will be used to make DNA, which is material containing your genetic information. The first IV may take up to 6 hours. It will be given in the hospital, clinic, or other healthcare setting, so we can watch you closely for any side effects. You will be monitored in the presence of a doctor throughout the first infusion.

< You may be asked to stay at the location of your study visit for 8 hours after your first IV began. Alternatively you may go home, but you will receive a follow-up phone call 4 to 8 hours after the end of this infusion to discuss any side effects you may have from the IV. THE CHOICE OF ONE OR BOTH OPTIONS IS CENTER SPECIFIC >. A nurse will also call you 16-24 hours after the end of your first IV.

After the first infusion, IVs will be given every 4 weeks until you deliver. They may take less time (3-5 hours) and will be given in the hospital, clinic, or other healthcare setting. We will collect blood and urine (one teaspoon each) before each monthly IV and store the samples in order to test the amount of the virus in your body as well as the response to the medication. We will also store some blood and urine in case we need to verify if you had any viruses before being given the study medication.

At each of these monthly visits, the study medication will be given by IV and nurses will ask you about any side effects or complications you may be experiencing or if you are taking any medication. A nurse will only call you 16-24 hours after the end of all IV sessions. You will continue to have study IVs until your baby is born. If you are admitted to the hospital for prenatal care, we will come to the hospital for the monthly study visits if possible. You will continue to receive standard care from your doctor while you are in this research trial.

When your baby is born, we will collect information on your pregnancy, labor, and the status of the baby. Your baby's head will be measured to record your child's growth. We may collect a small amount of blood (less than ¼ teaspoon) from your baby to check your baby's blood cells and liver. In the first few days after your baby is born, we will collect urine and saliva samples from your baby and send the samples to a lab to test if he or she is infected with CMV. We will tell you of the results of this test. We will also schedule a hearing test to take place before you leave if possible. If not, it will be scheduled within the first month to have specific hearing tests done on your baby. These hearing tests are very important to see if your baby has hearing loss since they include many more tests than the usual newborn hearing tests done in the hospital. We will tell you if your child has trouble hearing.

A nurse will keep in touch with you so that your child can have visits done at one and two years of age. At each visit, trained staff will collect information on medication he or she may be taking or medical events that occurred since the last visit. We will also collect another saliva sample from your child so that we can determine the amount of CMV virus (if any) is in your child's saliva. All of the tests that will be done are commonly used with young children. At the two year visit, we will collect a small amount of your baby's saliva to obtain DNA and trained staff will give your child hearing and developmental testing. If your child has hearing loss, you will be told after the test is completed. You will learn the results of the development testing after the 24 months visit.

Possible Risks

You may experience the discomforts associated with drawing blood or receiving the IV medication. This can include bruising, discomfort or pain at the site, infection, or fainting.

Both the active and placebo medication come from large pools of adult human blood. The effects on the fetus are unknown. Other antibodies like these are routinely used during pregnancy for a number of

reasons including exposure to other viruses (hepatitis B, chicken pox). Because Cytogam® and placebo are made from human blood it may carry a risk of transmitting infections. This is unlikely to happen because the CMV antibodies have been made from blood that has been carefully selected and tested and the creating of the drug involves removing viruses that cause infection.

Minor side effects associated with the infusion of CMV antibodies or protein in the placebo include flushing, chills, muscle cramps, back pain, fever, nausea, vomiting, joint pain and wheezing. These happen to about 6 out of every 100 infusions and are usually fixed by slowing the rate the medication is put into your body. Other serious side effects including aseptic meningitis syndrome (nerve swelling), lung injury, kidney failure, destruction of red blood cells, and blood clotting events, have been reported but the chance of these occurring is very small. In one study that was conducted in pregnant women, no side effects were reported with use of CMV antibodies. Another recent study suggests that there is a possible increase in preterm birth with treatment using CMV antibodies.

Other studies have been conducted using this placebo in pregnant women and no serious side effects were reported with use of the placebo. It is possible to have a severe allergic reaction or drop in blood pressure with the study infusion of CMV antibodies or placebo. If this happens, the infusion will be stopped immediately.

Although unlikely, it is possible that participation in this study could involve risks to you or your baby that are currently unexpected.

Benefits

If you decide to take part in this research study, you and your baby may not directly benefit from your participation. If the study shows that treatment with CMV antibodies prevents babies from being infected in women newly infected with CMV, this treatment may be made available to other pregnant women. Therefore, your participation can possibly help mothers and their babies in the future.

Consent for Use/Disposal of Blood, Urine and Saliva

By signing this consent form, you agree to the use of your blood and urine, and your baby's urine and saliva for testing the amount of virus in your blood or your baby's blood and for checking whether you had another virus before getting the first IV.

If you agree to have some of your blood sample processed to get DNA, we will test your DNA to see whether your genes change how the study medication works.

If any samples remain after the study tests, and if you agree, they may be used for future research, including DNA research, on the health of mothers and children. The samples, without information identifying you or your baby, will be sent to a National Institutes of Health sample storage facility, where they will be stored indefinitely. They will be accessed only by researchers approved by the National Institutes of Health. An Institutional Review Board must also approve any future research, including DNA research, using your and your baby's samples.

However if the researchers decide that there is no more use for your samples, you agree that they may be thrown away.

Alternative Procedures

The alternative to this study is not to participate and continue receiving standard monitoring and care during pregnancy, labor and delivery.

Costs

There will be no cost to you to take part in the research study. All research study related medications and procedures will be provided at no cost to you or your insurance company. The costs of your standard medical care will be billed to you or your insurance company in the usual manner.

Compensation

By signing this consent form, you acknowledge and agree that, in the event that this research project results in the development of any marketable product, you will have no ownership interest in the product and no right to share in any profits from its sale or commercialization.

(THIS SECTION WILL BE CENTER SPECIFIC.) You will be paid $XX to compensate you for the time and travel associated with the research study.

Payment for Injury or Harm

(THIS SECTION WILL BE CENTER SPECIFIC.) This medical institution and the NICHD have not made any provision for monetary compensation in the event of injury resulting from the research. In the event of such injury, treatment will be provided but it is not provided free of charge. Since this is a research study, payment for any injury resulting from your participation in this research study may not be covered by some health insurance plans.

Right to Withdraw From the Research Study

You are free to withdraw your consent and stop taking part in this research study at any time. Refusal to take part will involve no penalty or loss of benefits to which you are otherwise entitled. Nor will your refusal affect your legal rights or quality of health care that you will receive at this hospital. All of the information that has already been collected about you as part of the follow-up research study will continue to be used. No new information about you will be collected for research study purposes unless the information concerns an adverse event (bad effect) related to the follow-up research study.

Any significant new information which becomes available during your participation in this research, and which may affect your health, safety, or willingness to continue in this research study, will be given to you.

Right of the Investigator to Withdraw

The researchers of this institution or the National Institutes of Health can withdraw you from this study without your approval. A possible reason for withdrawal could be the early termination of the study by the National Institutes of Health.

Confidentiality

You have the right to privacy. All information obtained from this research that can be identified with you will remain confidential within the limits of the law.

The medical information collected on you for this research study will come from your medical record and from information you give the nurse, such as your previous pregnancies, height, weight, and whether you drink or smoke. Other information collected about you includes marital status, your level of education, type of medical insurance, potential sources of exposure to CMV, and current pregnancy complications. When your baby is born, data will be collected on your labor (such as when it starts) and delivery as well as information on your baby at birth and how your baby does in the hospital. Information will also be collected on your baby at one and two years of age. If we lose track of you, study staff may collect information from the internet including social network sites in order to find your contact information.

The information collected for this research study will be submitted to the data coordinating center (The George Washington University Biostatistics Center in Rockville, Maryland). There the information will be put into a database with information from all of the participants. Your information in the database will **only** be used for statistical analysis and may appear in scientific publications but will not identify you. The information sent to the data coordinating center does **not** include your name, address, social security number, hospital number, date of birth or any other personal identifiers. Instead, the data center will use a unique code for each person consisting of a number and the first letter of your first name. All samples

will be labeled with a unique barcode consisting of series of numbers. The key to the code linking the data and samples to you will be kept here in a locked file. Only the research study staff employed for this study at this hospital will have access to the key to the code.

The following individuals and/or agencies will be able to look at and copy your research records:

- The investigator, study staff and other medical professionals who may be evaluating the study.
- Authorities from this institution, including the Institutional Review Board (IRB) which is a group of people who are responsible for making sure the rights of participants in research are respected. Members or staff of the IRB at this medical center may also contact you about your experience with this research. You do not have to answer any questions the representative of the board may ask.
- The United States Food and Drug Administration (FDA) and/or the Office of Human Research Protections (OHRP)
- The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) which sponsors this study, including persons or organizations working with the sponsors, such as the data coordinating center, The George Washington University Biostatistics Center in Rockville, Maryland.

A copy of your medical chart also may be sent to research investigators at one of the other enrolling centers or the data coordinating center for review. If your chart is sent, all identifying information, such as your name, address, social security number, hospital number, and date of birth first will be removed. The results of this research study will be provided to the sponsor, NICHD (and/or their representatives).

Your data may be used by the company that makes the CMV antibodies and the FDA to decide whether other pregnant women can be offered CMV antibodies. The data will **not** include your name, address, social security number, hospital number, date of birth or other personal identifiers.

No information identifying you or your baby is on the samples used for the study. Your privacy is maintained by the use of a barcode label, the key to which is kept here in a locked file.

In addition, data from this study will be put in a public data set that will be available to other research investigators. This public data set will not contain any identifying patient data. If you agree to the use of your blood and urine for future research, samples will be sent with your data. These samples will be relabeled with a new code to make sure that they cannot be linked back to you.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

You will be told the results of your baby's CMV test, hearing tests, and developmental tests. These tests are being done only because you are in this study. The results will not be sent to your physician to include in your medical record.

This permission does not end unless you cancel it, even if you leave the study. You can cancel this permission any time except where a healthcare provider has already used or released your health information, or relied on your permission to do something. Even if you cancel this authorization, the researchers may still use and disclose protected health information (PHI) they already have obtained about you as necessary to maintain the integrity or reliability of the research. However, no new PHI or new biological specimens will be collected from you after you revoke your authorization.

To cancel your authorization, you will need to send a letter to Dr. _____ of the _____ stating that you are canceling your authorization. This letter must be signed and dated and sent to this address:_____. If you are unable to write a letter ask one of the research staff to provide you with a letter that must be signed, dated, and sent to the above address. A copy of this revocation will

be provided to the Study Doctor and his or her research team. Not signing this form or later canceling your permission will not affect your health care treatment outside the study, payment for health care from a health plan, or ability to get health plan benefits.

Your protected health information will be treated confidentially to the extent permitted by applicable laws and regulations. Federal law may allow someone who gets your health information from this study to use or release it in some way not discussed in this section and no longer be protected by the HIPAA Privacy Rule.

By signing this form you authorize the Study Doctor and members of the research team to use and share with others (disclose) your PHI for the purpose of this study. If you do not wish to authorize the use or disclosure of your PHI, you cannot participate in this study because your PHI is necessary to conduct this study.

Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality from the NIH. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless you have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings); if you have consented to the disclosure, including for your medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the NIH or for information that must be disclosed in order to meet the requirements of the FDA. You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

Questions

The researchers are available to answer your questions about this research. A representative of the Institutional Review Board is also available to answer questions about your rights as a participant in research or to answer your questions about an injury or other complication resulting from your participation in this research study.

If you have questions or are hurt while taking part in this research study, you should contact _____ at (___) ___-____.

If you have any questions about the informed consent process or any other rights as a research subject, please contact _____, at (____) ___-____. _____ .

Signatures

By signing below, you indicate that you have read this consent form, the study has been explained to you, your questions have been answered, and you agree to take part in this study. You do not give up any of your legal rights by signing this form. A copy of this consent form will be given to you.

Please initial below to indicate whether or not you give permission for the study team to get DNA from *your* blood sample drawn at the first IV.

YES_____ I **agree** to have some of my blood sample made into DNA and used to see how genetic factors affect how the study medication works.

NO_____ I **do not agree** to have some of my blood sample made into DNA and used to see how genetic factors affect how the study medication works.

Please initial below to indicate whether or not you give permission for future research, including DNA research, of *your* samples.

YES_____ I **agree** to have my blood, urine, and DNA, which will be kept confidential, stored and shared with other investigators doing research which is related to CMV or the health of mothers and children.

NO_____ I **do not agree** to have my blood, urine, and DNA, which will be kept confidential, stored and shared with other investigators doing research which is related to CMV or the health of mothers and children.

Please initial below to indicate whether or not you give permission for future research, including DNA research, of *your baby's* samples.

YES_____ I **agree** to have my baby's urine, saliva, and DNA, which will be kept confidential, stored and shared with other investigators doing research which is related to CMV or the health of mothers and children.

NO_____ I **do not agree** to have my baby's urine, saliva, and DNA, which will be kept confidential, stored and shared with other investigators doing research which is related to CMV or the health of mothers and children.

The investigator or study team may wish to contact you in the future to request permission for additional research. Please initial the appropriate statement to indicate whether or not you give permission for future contact.

YES_____ I **give** permission to be contacted in the future for follow-up research.

NO_____ I **do not give** permission to be contacted in the future for follow-up research.

_____	_____	_____
Signature of Mother	Signature	Date
(Print Name)		
_____	_____	_____
Signature of Father	Signature	Date
(Print Name)		
_____	_____	_____
Person Obtaining Consent	Signature	Date
(Print Name)		

ASSENT FOR FEMALES UNDER 18 YEARS of AGE (if required by Center IRB):

I agree _____ I do not agree_____ to participate in this study.

This has been explained to me by _____.

_____ _____

Signature of Minor Date

_____ _____

Print Name of Subject Age

Please provide either one or both parental signatures as instructed by your IRB.

_____ _____

Signature of Mother/Guardian Date

_____ _____

Signature of Father/Guardian Date

A witness unrelated to the study is necessary if the participant can comprehend but cannot read (e.g., blind), or cannot sign (e.g., unable to use hands) the consent form.

_____ _____ _____

Witness' Name Signature Date

(Print Name)

Investigator Statement

I certify that the research study has been explained to the above individual by me or my research staff including the purpose, the procedures, the possible risks and the potential benefits associated with participation in this research study. Any questions have been answered to the Individual's satisfaction.

_____ _____ _____

Investigator Signature Date

(Print Name)

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