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Safety and Efficacy of a Cytomegalovirus Glycoprotein B (gB) Vaccine in Adolescent Girls: a Randomized Clinical Trial

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Abstract

Background—Cytomegalovirus (CMV) is a leading cause of congenital infection and an important target for vaccine development.

Methods—CMV seronegative girls between 12 and 17 years of age received CMV glycoprotein B (gB) vaccine with MF59 or saline placebo at 0, 1 and 6 months. Blood and urine were collected throughout the study for evidence of CMV infection based on PCR and/or seroconversion to non-vaccine CMV antigens.

Results—402 CMV seronegative subjects were vaccinated (195 vaccine, 207 placebo). The vaccine was generally well tolerated, although local and systemic adverse events were significantly more common in the vaccine group. The vaccine induced gB antibody in all vaccine recipients with a gB geometric mean titer of 13,400 EU; 95% CI 11,436, 15,700, after 3 doses.

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Conflict of Interest

DIB has consulted about herpes vaccines with Merck Inc. and is evaluating CMV vaccines for Hookipa Biotech AG, KE is Chair of DSMB for Novartis influenza study, SP is an employee of Sanofi Pasteur, all others no conflicts.

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Overall, 48 CMV infections were detected (21 vaccine, 27 placebo). In the per protocol population (124 vaccine, 125 placebo) vaccine efficacy was 43%; 95% CI: -36; 76, P=0.20. The most significant difference was after 2 doses, administered as per protocol; vaccine efficacy 45%, 95% CI: -9; 72, P=0.08.

Conclusion—The vaccine was safe and immunogenic. Although the efficacy did not reach conventional levels of significance, the results are consistent with a previous study in adult women (Pass et al NEJM 360:1191, 2009) using the same formulation.

Keywords

cytomegalovirus; vaccine; adolescent; CMV gB

Introduction

Cytomegalovirus (CMV) is a significant pathogen in congenital infections and in immunocompromised patients. Between 0.5 and 2.0% of infants worldwide are congenitally infected with CMV, including about 0.64% in Western countries [1]. In the United States (US), congenital CMV infections account for about 400 deaths and 5,000–8,000 significantly impaired children each year [2, 3]. It is the most common viral cause of sensorineural hearing loss (SNHL) and developmental delay in the country [4, 5]. In 2000, the U.S. Institute of Medicine issued a report that listed a CMV vaccine to prevent congenital infections as the highest priority based on cost savings and health benefits [6]. In 2012 a multidisciplinary meeting was held to discuss priorities related to development of CMV vaccines [7, 8].

Multiple approaches to the development of CMV vaccines have been evaluated including live attenuated, plasmid DNA, viral-vectored, and subunit vaccines (reviewed in [9, 10]). Most recently, two vaccines have been evaluated in transplant patients. A plasmid DNA vaccine coding for pp65 and gB with a poloxamer adjuvant was found to reduce CMV viremia in hematopoietic cell transplant patients [11] while a subunit gB vaccine administered with MF59 as an adjuvant reduced the duration of CMV viremia and the duration of antiviral therapy [12]. The gB subunit vaccine also provided modest (50%) protection in preventing CMV infection in young women [13].

The most important reason for developing a CMV vaccine is to prevent congenital CMV disease. One recognized strategy for the prevention of congenital CMV is to immunize adolescent girls, or perhaps both boys and girls, before the onset of sexual activity as sexual activity is an important mode of transmission after infancy and the toddler years [7, 8]. This trial evaluated the gB/MF59 vaccine in adolescent girls.

Methods

Participants and study design

This study was a randomized, double-blind, placebo-controlled, Phase II study designed to assess the safety and efficacy of the experimental CMV gB/MF59 vaccine in healthy adolescent females. Healthy females, age 12 to 17 years at time of screening, were recruited

from 5 sites in the USA in order to obtain approximately 400 CMV-seronegative subjects for the vaccine trial (N=200 per group). Enrollment began on July 26, 2006 and the last subject visit was conducted on June 10, 2013. After signing the screening consent and parental consent (if subject was <18 years old), subjects were screened for antibodies to CMV. Subjects who were CMV-seronegative then consented to participate in the vaccine study (with parental consent if <18 years old) and were randomized 1:1 to receive either the vaccine or saline placebo. The randomization sequence used permuted blocks (randomly selected block size of 4 or 8). The randomization list was available only to the unblinded pharmacist and vaccine administrator. All other site staff, subjects, and laboratory staff were blinded to the treatment assignment. In order to participate, subjects had to be using an effective method of birth control if they were sexually active. Subjects also could not be receiving or have a history of receiving any medications or treatments that affected the immune system, could not have received a blood transfusion or blood products within 3 months, or have active or previous drug abuse. A complete description of the inclusion/exclusion criteria can be found in the supplemental table 1. Subjects received 3 doses of vaccine or saline placebo administered by intramuscular (IM) injection in the deltoid muscle on a 0-, 1-, and 6-month schedule. Collection of sera occurred at screening, study day 0, month 6, month 7 and every three months for two years after month 7 for the analysis of CMV shedding by polymerase chain reaction (PCR) and for assessment of seroconversion to non-vaccine CMV antigens by a gB adsorption assay. Collection of urine occurred at study day 0, month 1, month 2, month 6, month 7 and every three months for two years after month 7 for the assessment of CMV shedding by PCR.

If subjects seroconverted or had CMV detected by PCR at any time on or after Day 0 of the study, they were eligible to enter the shedding portion of the study. The subject and her parent (if subject was <18 years old) signed another informed consent detailing this portion of the study. The shedding sub study included monthly visits for 4 months to obtain urine, saliva, and blood samples for quantification of CMV by PCR and then every other month for 8 months for the same purpose.

Study objectives

The primary objective for the efficacy analysis was protection from a systemic infection, defined as identification of CMV from the urine or blood evaluated by PCR. The secondary efficacy objective was protection from CMV infection, defined as systemic infection or seroconversion to non-vaccine CMV antigens. The primary safety outcomes were the incidence of local and systemic reactions within 7 days of vaccination, adverse events (AEs) occurring within the 30-day period (± 2 days) after vaccination, and serious adverse events (SAEs) observed at any time throughout the study.

Additional secondary outcomes included CMV shedding in infected subjects measured by the duration and magnitude of CMV replication in the urine and blood and CMV antibody measurements by CMV gB enzyme-linked immunoassay (ELISA).

Vaccine

CMV gB/MF59 is a subunit gB glycoprotein of human CMV (strain Towne) expressed in Chinese Hamster Ovary (CHO) cells. The CMV gB/MF59 vaccine (20 µg CMV glycoprotein gB and 10.75 mg MF59) was provided by Sanofi Pasteur as 2 separately vial components, which were combined prior to administration. The 20 µg dose was based on prior evaluations [14]. Sterile Saline (Sodium Chloride 0.9%) was used as the placebo.

Assessment of safety

Local and systemic signs and symptoms including temperature were collected the day of vaccination and for 6 follow-up days after each vaccination, recorded by the subject on a memory aid. For thirty days after each dose, all AEs were collected. SAEs were collected for the duration of the study. A Safety Monitoring Committee monitored the study progress and addressed any specific safety concerns. Unsolicited AEs were coded to Medical Dictionary for Regulatory Activities Terminology (MedDRA) version 14.0 or later terms.

Assessment of immunogenicity

Blood for immunogenicity testing was obtained at baseline, prior to the third vaccination at 6 months, and at months 7, 13, 19, 25 and 31.

Laboratory Tests

Screening for Anti- CMV IgG antibody—Anti-CMV IgG antibodies were detected by use of the Wampole CMV IgG enzyme immunoassay (EIA) kit (Fisher Scientific, Pittsburg, PA). This assay was performed, at a central site, according to the manufacturer's instructions to determine if the subject was eligible for the study.

CMV gB IgG ELISA Assay to determine CMV anti gB antibody response—IgG antibody titers to gB were measured using an ELISA assay developed and validated at Cincinnati Children's Hospital Medical Center (CCHMC). Serum antibody levels were determined using 0.75 µg/mL gB (vaccine preparation) diluted in coating buffer (pH 9.5) to coat plates overnight at 4°C. After plates were washed with phosphate-buffered saline plus 0.05% Tween 20 (wash buffer), plates were blocked using 0.89% Bovine Serum Albumin (Sigma, St. Louis, MO) in wash buffer. A human gB positive serum, assigned an arbitrary value of 5,120 units was used to generate a standard curve by starting at a dilution of 1:400. Samples were run in a series of two fold dilutions. After washing the blocking solution from the plates, standards and samples were added to wells and incubated for 45 minutes at 37°C. After washing, peroxidase conjugated goat anti-human IgG (KLP, Inc., Gaithersburg, MD) was added and incubated for 30 min at 37°C. Plates were washed and 3,3',5,5'-Tetramethylbenzidine (TMB), peroxidase substrate system (KLP, Inc.) was added for 30 min at room temperature. The reaction was stopped by adding 1.0M H₃PO₄ and the A₄₅₀ was read on a Molecular Devices SpectraMax ELISA reader. The average OD values of the triplicate wells of the standard were plotted using a 4 parameter best fit method and used to calculate the ELISA Units (EU) of gB antibody. The lower limit of quantitation of the assay was 15 units.

Detection of CMV; CMV PCR—Nucleic acid isolation from subject specimens was performed using either the Qiagen QIAamp DNA Blood extraction system (all specimens except urine) or the Qiagen QIAamp Viral RNA Mini Kit (urine specimens) as specified by the manufacturer (Qiagen, Valencia, CA). CMV detection was determined by real-time PCR using previously described methods and primers [15, 16]. Specific 5' and 3' primer sequences and hybridization probes were obtained from Integrated DNA Technologies (Coralville, IA). Two sets of primers and probes were used to simultaneously amplify a 68 bp of the gB region (UL 55) and 84 bp of UL123-exon 4. As an internal control, EXO DNA derived from a Jellyfish DNA sequence, was added to each reaction to determine if the PCR reaction was inhibited by nonspecific factors. Each PCR reaction contained 400 nM of each primer and 100nM probe, Applied Biosystems 2X PCR Master mix (Life Technologies, Grand Island, NY) and 5 µl of the extracted DNA in a total volume of 25 µl. Real-time PCR was performed using an Applied Biosystems 7500 PCR machine and software. The temperature cycling profile began with an initial 50°C for 2 min followed by 95°C for 10 min, then 95°C for 20 seconds, 60°C for 2 min for 45 cycles. To be positive for CMV detection, a sample had to have a Ct value <45. CMV culture material (Towne strain, ATCC #VR-977) and nuclease-free water were used as positive and negative controls, respectively.

For subjects enrolled in the shedding study, a quantitative real-time PCR assay was established using the same primers and probes but using a standard curve generated from a commercially available preparation of quantitated CMV DNA (Advanced Biotechnologies, Inc., Columbia, MD). Urine, saliva and plasma samples were analyzed using this assay. Data for CMV-positive samples were reported as genome copies/mL of bodily fluid, with a reportable limit of 50 genome copies/mL of bodily fluid.

Statistical Analysis

Sample size estimates were based on previous studies showing an annual 13% to 14% risk of CMV infection in adolescence girls at CCHMC [17]; however, the annual risk was unknown for the other clinical sites. Therefore the study was designed using a conservative attack rate of 10% per year (20% over 2 years). The study design assumed a 60% vaccine efficacy (VE) and a dropout rate of 12% per year yielding a sample size of 350 subjects to have 80% power to detect a difference of 0.12 for the proportions surviving uninfected in the placebo group and the CMV gB/MF59 vaccine group (0.80 and 0.92, respectively) using a 2-sided log-rank at a 0.05 significance level. Target enrollment was later increased to 400 subjects due to a higher than expected dropout rate (per year) and attack rates that were below initial expectations.

For each outcome measure, VE was analyzed using a Cox proportional hazards model, and cumulative incidence curves (1 - Kaplan-Meier) were compared between treatment groups using the log-rank test. The p-value obtained from the log-rank test was used as the primary determination for statistical significance of the primary outcome measure. Additionally, for each outcome measure, attack rates were compared using a Fisher's Exact test, and number of events per 100 person years with 95% confidence interval (CI) were estimated.

The safety analyses included all randomized subjects who received at least one dose of study product and had at least one post-baseline safety assessment. Analysis populations were

defined to evaluate efficacy after each of the 3 doses. Three per-protocol (PP) populations were comprised of subjects who received one PP-1 (N=381), two (PP-2, N=336), or three doses (PP-3, N=249). To be included in PP analysis for the specified dose, subjects must have received all vaccinations up to and including that dose within the protocol defined window, must not have had CMV infection prior to or within 20 days after the dose, and must not have had any significant protocol deviations occurring before that dose. The intent-to-treat after 1 dose (ITT-1, N=400) population was comprised of all subjects who received at least one dose of study product and were free of CMV infection prior to or within 20 days after the first dose of product. Similarly the ITT-2 (N=385) and ITT-3 populations (N=349) received at least 2 or all 3 doses and were free of CMV infection prior to or within 20 days after the second or third dose, respectively.

The immunogenicity analyses include all subjects who received at least two doses of study product and had at least one post-baseline immunogenicity assessment. The shedding analyses include subjects who seroconverted or had CMV detected on or after Day 0 and provided at least 1 day of results from quantitative PCR analysis. Analysis of data from the shedding sub-study was primarily descriptive including summaries of the number of shedding study visits with PCR analysis, the number of visits with detectable CMV shedding, the initial duration (days) and overall duration (days) with detectable CMV shedding, and the maximum observed CMV replication for the shedding population.

All statistical analyses were performed in SAS version 9.2 or higher. All tests are two-sided without adjustment for multiple comparisons.

Results

Study population

A total of 2137 subjects were screened to participate in the study and 409 eligible subjects were enrolled in the vaccine study; 402 subjects were vaccinated (Table 1). Thirty-two subjects subsequently enrolled in the shedding sub-study and provided specimens. Subjects were 49% Caucasian, 41% Black and predominantly non-Hispanic with an average age of 14.6 years (Table 2). Demographics were similar between groups and similar comparing all vaccinated subjects (Table 2) to the per protocol populations (not shown). A table summarizing the analyzed populations is provided as supplement 2.

Safety

Overall the vaccine appeared safe, although there was a significant increase in local injection site reactions during the 7 day reactogenicity period (Figure 1). Local injection site reactions after any vaccine dose were reported in 92% (95%CI: 87–95%) of vaccine recipients compared to 64% (95%CI: 57–71%) of placebo recipients (p-value<0.001). All but two local injection site reactions were mild or moderate; the two severe local reactions occurred after the third dose in the CMV gB/MF59 group. One of these subjects reported severe pain at injection site on Day 3 while the other subject reported severe induration on the day of vaccination through Day 1. Pain was the most commonly reported local reaction in both the CMV gB/MF59 and placebo groups.

Systemic reactions were less common than local reactions but also occurred more frequently in the CMV gB/MF59 group (82%, 95%CI: 75–87%) than placebo recipients (67%, 95%CI: 60–74%; p-value=0.001). Fatigue and headache were the most frequently reported systemic event in the CMV gB/MF59 group. Fatigue was the most frequently reported systemic event in the placebo group. Fever (temperature >100.2) was detected in 9% (95%CI: 5–14%) of vaccine and 3% (95%CI: 1–7%) of placebo recipients (p-value=0.034). Severe systemic events were reported by 5 subjects following the first vaccination (2 vaccinees, 1 with severe fatigue, 1 with severe fatigue and myalgia; 3 placebo subjects: 2 with severe headache, 1 with severe fatigue and myalgia); 4 subjects following the second vaccination (2 vaccinees: 1 with severe headache, 1 with severe fatigue and myalgia; 2 placebo: 1 with severe headache, 1 with severe fatigue and headache); and 5 subjects following the third vaccination (4 vaccinees: 2 with severe fatigue and headache, 1 with severe fatigue, headache and myalgia, 1 with severe myalgia, 1 placebo with severe myalgia). Overall, the proportions of subjects reporting local and systemic reactions did not appear to increase after the second or third injections. There were no subjects who discontinued the study early due to an AE or SAE.

Unsolicited AEs were reported by 277 (69%) of subjects during the study; 72% (95%CI: 66–79%) in the vaccine and 66% (95%CI: 59–72%) in the placebo group (p-value=0.162). Gastrointestinal disorders were more common in the vaccine group; 22% (95%CI: 16–28%) vs. 11% (95%CI: 7–16%) in the placebo group (p-value=0.006) as were nervous system disorders 15% (95%CI: 10–21%) vs. 8% (95%CI: 5–13%) respectively (p-value=0.041). However, no concerns were noted for the distribution of AEs within these classifications and no single type of AE was significantly more common in the vaccine group. Thirty SAEs were reported in 24 subjects; 16 (12 subjects) in the vaccine and 14 (12 subjects) in the placebo group. None of the SAEs were associated to the study product.

Immunogenicity

The gB/MF59 vaccine was immunogenic (Figure 2). Approximately 5 months after the second dose of vaccine (the first post vaccine measurement), all gB/MF59 vaccinated subjects had developed a 10-fold antibody increase to gB as measured by ELISA. A 10-fold increased response remained for 2 years after the third dose of vaccine in all but one subject. A substantial increase in the GMT of gB ELISA antibody from GMT of 1204 EU/mL (95%CI: 1075–1349) at 5 months after dose 2 (just before dose 3) to GMT of 13,400 EU/mL (95%CI: 11,436–15,700) at 1 month post dose 3. The GMT remained high, 2259 EU/mL (95%CI: 1985–2572) at one year and 1453 EU/mL (95%CI: 1252–1685) at two years after the third dose of vaccine. As a comparison the peak gB antibody GMT was 2162 (95%CI: 1026–4554) for placebo recipients that became infected during the study.

Efficacy

Efficacy was evaluated for both PP and ITT populations after each dose (Table 3). Efficacy was evaluated against systemic CMV infection and for all CMV infections. Because the proportion of systemic to total infections was similar between vaccine and placebo groups; for example, in the ITT-1 population 75% and 76%, respectively, only total infections are discussed. None of the analyses revealed a statistically significant difference in the attack

rate between vaccinees and placebo recipients, although for each analysis, the attack rate was lower in the vaccine group (Table 3). Evaluation after 3 doses in the PP population (Figure 3A) revealed 14 infections in 125 placebo recipients compared to 8 infections in 124 vaccinees; VE from a Cox proportional hazards model was 43% (95% CI –36, 76; p-value=0.200). Similar estimates of VE were obtained for the PP population using the observed attack rates (efficacy 42% [95% CI: –32, 75%]) (Fisher's exact test, p-value = 0.264) (data not shown). The ITT analysis revealed a VE from a Cox proportional hazards model of 23% (95%CI: –57, 62%) after 3 doses.

In the ITT population, after one dose the VE was 19% (95% CI: –44–54%). After two doses, the VE was 15% (95%CI: –53, 53%) in the ITT population. Considering only PP subjects, the VE after two doses was 45% (95%CI: –9, 72%) (Figure 3B). In this population: 13 of 164 in the vaccine group compared with 24 of 172 placebo recipients developed an infection (Table 3, p-value=0.082).

The analysis of shedding in 32 CMV infected subjects (16 vaccinees and 16 placebo recipients) did not reveal any apparent differences in either the magnitude or duration of CMV shedding. For example the peak shedding from any sight was 5.8×10^5 genome copies/mL for vaccine and 1.8×10^5 genome copies/mL for placebo recipients while the duration of CMV shedding was (223 and 212 days) for vaccine and placebo recipients respectively (data not shown).

Discussion

Cytomegalovirus is the most common cause of congenital infection in the developed world. Each year in the US, an estimated 5,000 to 8,000 infants suffer disabilities, including mental retardation and sensorineural deafness, as a result of congenital CMV infection [2–4]. Since preconceptual maternal antibody to CMV helps to protect against the sequelae of congenital infection, there is interest in the development of CMV vaccines [2, 18–20]. The subunit vaccine evaluated here is a recombinant form of gB protein expressed in Chinese hamster ovary (CHO) cells. It contains the entire gB coding sequence, except for the transmembrane domain, which was deleted to facilitate secretion. In addition, the furin cleavage site has been mutagenized, resulting in secretion of gB as an uncleaved protein. The recombinant gB, 807 amino acids in length, is formulated with a proprietary experimental adjuvant, MF59 (10.75 mg/dose), a squalene-in-water emulsion.

Overall the vaccine appeared safe, although as expected, there was a significant increase in local injection site and systemic reactions in the vaccine recipients compared to placebo recipients. Local injection site reactions after any vaccine dose were reported in 92% of vaccine recipients compared to 64% of placebo recipients (p-value<0.001) while systemic reactions were reported in 82% vs. 67% of vaccine and placebo recipients respectively (p-value=0.001). Unsolicited AEs were reported by 277 (69%) of subjects during the study; 72% in the vaccine and 66% in the placebo group (p-value=0.162). No patterns or concerns were noted for the distribution of AEs.

Similar to previous studies, the CMV gB/MF59 vaccine was immunogenic [13, 14, 21]. Approximately 5 months after the second dose of vaccine (the first post vaccine measurement) all gB/MF59 vaccinated subjects had developed a 10-fold increased antibody response to CMV gB as measured by ELISA. This was boosted a further 10-fold by the third dose of vaccine. Titers remained above the level detected after the second dose for two years. Antibody data from previous studies have shown three doses of vaccine given in the same schedule as the study reported here induced levels of CMV gB antibody that were several times higher than those in adults with past CMV infection. These previous studies also showed that the peak level of neutralizing antibody that occurs after the third dose of vaccine was similar to that following naturally acquired infection.

Efficacy was assessed for several analysis populations and after at least 1, 2 or 3 doses. None of the analyses revealed a significant difference in attack rate between vaccinees and placebo recipients although, for each analysis, the attack rate was lower in the vaccine group. Evaluation for protection from CMV infection after 3 doses in the PP population, revealed a VE from a Cox proportional hazards model of 43% (95% CI: -36, 76%; p-value=0.200). Similar estimates of VE were obtained using the observed attack rates; efficacy 42% (95% CI: -32, 75%). The largest difference in attack rates was seen after 2 doses in the PP population, VE 45% (95% CI: -9, 72%; p-value=0.082). Analysis was somewhat limited by the lower than expected attack rate which produced wide confidence intervals. Initial sample size estimates were based on a study conducted in Cincinnati [17], but attack rates were substantially lower in the other sites.

The efficacy detected in the trial reported here was less than but consistent with those obtained during a previous evaluation of the same vaccine in a slightly older female population [13]. In the previously reported study of 464 CMV seronegative women vaccinated one year after giving birth, the VE was 50% (95% CI: 7, 73%) on the basis of infection rates per 100 person-years. Congenital CMV infection occurred in 1/81 (1%) and 3/97 (3%) babies born to CMV gB vaccine and placebo recipients, respectively. These numbers were too small to allow any conclusions regarding the vaccine's ability to prevent congenital infection beyond its efficacy for prevention of maternal infection. Infants born during the trial presented here were not assessed for CMV infections, therefore no additional information on this important outcome is provided.

Despite the induction of high anti gB titers the vaccine appears to be only marginally protective. There are several possibilities for this including that the antibody induced is poorly functional or limited in breadth by CMV strain differences, or that cell mediated immune responses are needed for protection but were not induced at the needed level. It is also possible that a CMV vaccine will be more effective in preventing congenital infections or congenital disease than maternal infections but this hypothesis will require large phase 3 trials. In the guinea pig model of congenital CMV, gB vaccines have indeed been more effective in preventing congenital infection than maternal infection [22].

Although this candidate vaccine appeared to provide some protection in the study reported here, and significant protection in the evaluation by Pass et al [13] the efficacy was not sufficient to continue the development of this formulation as a standalone agent for the

prevention of CMV infection. We believe that before proceeding to difficult and expensive efficacy studies to prevent congenital CMV infections, more effective vaccines need to be developed. It may be possible to add other CMV antigens to this vaccine to either improve neutralizing activity or T cell mediated immunity, or it may be necessary to use other vaccine strategies to improve efficacy [10, 23].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- CMV seronegative girls between 12 and 17 years of age received CMV glycoprotein B (gB) vaccine with MF59 or saline placebo at 0, 1 and 6 months
- The vaccine was generally well tolerated, although local and systemic adverse events were significantly more common in the vaccine group
- In the per protocol population vaccine efficacy was 43% after 3 doses, $P=0.20$ and 45%, $P=0.08$ after 2 doses.
- We conclude the vaccine was safe and immunogenic and although the efficacy did not reach conventional levels of significance, the results are consistent with a previous study in adult women (Pass et al NEJM 360:1191, 2009) using the same formulation.

Figure 1. Safety analysis following any vaccination

This includes all subjects who received at least one dose of study product. The percent of subjects reporting each solicited injection site reaction or systemic symptoms following any of the vaccine doses are shown along with the 95% CI.

Figure 2. The GMT of CMV gB ELISA titers

Serum was obtained before, and one month after the third immunization, and then every 6 months for 2 years after the third vaccination, and antibody to CMV gB measured by ELISA. All subjects who received at least 2 doses of vaccine and had at least 1 post-baseline ELISA result were included. Subjects were censored after acquiring CMV infection.

A) After 3 Doses Per Protocol Population

B) After 2 Doses Per Protocol Population

Figure 3. Cumulative Incidence of CMV infections

Subjects were followed for evidence of CMV infection by PCR analysis of urine or seroconversion to non-vaccine CMV proteins for 3 years. Vaccine efficacy was analyzed using a Cox proportional hazards model, and cumulative incidence curves (1 - Kaplan-Meier) were compared between treatment groups using the log-rank test. Figure A includes the per protocol population after 3 doses of the vaccine or placebo. Figure B includes the per protocol population who received 2 doses of the vaccine or placebo.

Table 1

Disposition of All Subjects

	CMV gB + MF59 (N=195) n (%)	Placebo (N=207) n (%)	All (N=402) n (%)
Number of Subjects Screened	-	-	2137
Number of Subjects Enrolled/Randomized	200	209	409
Number of Subjects Enrolled and Not Vaccinated	5	2	7
Number of Subjects Receiving 1st Dose	195 (100.0)	207 (100.0)	402 (100.0)
Number of Subjects Receiving 2nd Dose	187 (95.9)	200 (96.6)	387 (96.3)
Number of Subjects Receiving 3rd Dose	171 (87.7)	186 (89.9)	357 (88.8)
Number of Subjects Completing the Study ^[1]	174 (89.2)	179 (86.5)	353 (87.8)
Number of Subjects Entering Shedding Sub-study	16 (8.2)	18 (8.7)	34 (8.5)
Number of Subjects Discontinuing Early from the Study	23 (11.8)	23 (11.1)	46 (11.4)
Reasons for Early Discontinuation from the Study			
Non-compliance/Protocol deviation	1 (0.5)	-	1 (0.2)
Lost to follow-up	13 (6.7)	12 (5.8)	25 (6.2)
Voluntary Withdrawal by Subject or parent/guardian	3 (1.5)	9 (4.3)	12 (3.0)
Withdrawal by Investigator	1 (0.5)	-	1 (0.2)
Randomized but not Vaccinated/Dosed	5 (2.6)	2 (1.0)	7 (1.7)
Number of Subjects in the Safety Population	195 (100.0)	207 (100.0)	402 (100.0)
Number of Subjects in the Intent-to-Treat after 1 Dose Population(ITT-1)	195 (100.0)	205 (99.0)	400 (99.5)
Number of Subjects in the Per Protocol after 1 Dose Population (PP-1)	189 (96.9)	192 (92.8)	381 (94.8)
Number of Subjects in the Intent-to-Treat after 2 Doses Population(ITT-2)	186 (95.4)	199 (96.1)	385 (95.8)
Number of Subjects in the Per Protocol after 2 Doses Population(PP-2)	164 (84.1)	172 (83.1)	336 (83.6)
Number of Subjects in the Intent-to-Treat after 3 Doses Population (ITT-3)	168 (86.2)	181 (87.4)	349 (86.8)
Number of Subjects in the Per Protocol after 3 Doses Population (PP-3)	124 (63.6)	125 (60.4)	249 (61.9)
Number of Subjects in the Immunogenicity Population	180 (92.3)	192 (92.8)	372 (92.5)
Number of Subjects in the Shedding Population	16 (8.2)	16 (7.7)	32 (8.0)

Denominator for percentages is the number of subjects in the Safety Population for each group.

^[1] Subjects counted as completing the study completed the Final visit that occurred 25 months after the first dose. Three subjects completed the shedding study but did not complete the final visit that occurred 25 months after their first dose.

Table 2

Demographics for Enrolled Subjects Receiving at Least One Dose

	CMV gB + MF59 (N=195)	Placebo (N=207)	All (N=402)	P-value
N Subjects in Safety Population	195	207	402	
Age at Vaccination(years)				0.1890 ^[1]
Mean (SD)	14.7 (1.64)	14.5 (1.60)	14.6 (1.62)	
Median	15.0	15.0	15.0	
Min, Max	12, 18	12, 17	12, 18	
Q1 : Q3	13.0 : 16.0	13.0 : 16.0	13.0 : 16.0	
Age Category at Vaccination, n (%)				0.5860 ^[2]
12–15 years old	124 (64)	137 (66)	261 (65)	
> 15 years old	71 (36)	70 (34)	141 (35)	
Ethnic origin, n (%)				0.5956 ^[2]
Hispanic or Latino	22 (11)	20 (10)	42 (10)	
Not Hispanic or Latino	173 (89)	187 (90)	360 (90)	
Race, n (%)				0.2494 ^[2]
Black	77 (39)	88 (43)	165 (41)	
Caucasian	103 (53)	95 (46)	198 (49)	
Other	15 (8)	24 (12)	39 (10)	

^[1] P-value is from an analysis of variance with treatment as an effect in the model.

^[2] P-value is from a Chi-square test.

Table 3

Vaccine Efficacy

	CMV gB/MF59		Placebo		N	Vaccine Efficacy (%) [1]	95% CI for Vaccine Efficacy [2]	P-value [3]
	n	CMV infection (%)	n	CMV infection (%)				
CMV Infection after 3 Doses								
Per Protocol after 3 Doses	8 (6.5%)	124	14 (11.2%)	125	42.9	-36.2, 76.0	0.200	
Intention to Treat after 3 Doses	13 (7.7%)	168	18 (9.9%)	181	23.2	-56.7, 62.4	0.466	
CMV Infection after 2 Doses								
Per Protocol after 2 Doses	13 (7.9%)	164	24 (14.0%)	172	44.5	-8.9, 71.8	0.082	
Intention to Treat after 2 Doses	20 (10.8%)	186	25 (12.6%)	199	15.0	-53.1, 52.8	0.589	
CMV Infection after 1 Dose								
Per Protocol after 1 Dose	18 (9.5%)	189	26 (13.5%)	192	30.7	-26.4, 62.0	0.229	
Intention to Treat after 1 Dose	21 (10.8%)	195	27 (13.2%)	205	18.5	-44.1, 53.9	0.480	

Note: N = Number of subjects in specific treatment group. n = Number of subjects with CMV infection.

[1] Vaccine efficacy is obtained from Cox regression.

[2] 95% CI = 95% confidence interval obtained from Cox regression.

[3] P-value = result of comparison of Kaplan-Meier survival curves between groups by Log-rank test.