Official Protocol Title:	Double-Blind, Randomized, Placebo-Controlled Phase 2b, Multi-center Study to Evaluate the Safety, Tolerability, Efficacy and Immunogenicity of a 2-Dose and a 3-Dose Regimen of V160 (Cytomegalovirus [CMV] Vaccine) in Healthy Seronegative Women, 16 to 35 Years of Age
NCT number:	NCT03486834
Document Date:	07-MAY-2019

Title Page

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Protocol Title: Double-Blind, Randomized, Placebo-Controlled Phase 2b, Multi-center Study to Evaluate the Safety, Tolerability, Efficacy and Immunogenicity of a 2-Dose and a 3-Dose Regimen of V160 (Cytomegalovirus [CMV] Vaccine) in Healthy Seronegative Women, 16 to 35 Years of Age

Protocol Number: 002-02

Compound Number: V160

Sponsor Name and Legal Registered Address:

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc.
(hereafter referred to as the Sponsor or Merck)

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Regulatory Agency Identifying Number(s):

IND NUMBER: [15778]

EudraCT NUMBER: [2017-004233-86]

Approval Date: 07 May 2019

Sponsor Signatory

Typed Name: Date
Title:

Protocol-specific Sponsor Contact information can be found in the Investigator Trial File Binder (or equivalent).

Investigator Signatory

I agree to conduct this clinical trial in accordance with the design outlined in this protocol and to abide by all provisions of this protocol.

Typed Name: Date
Title:

DOCUMENT HISTORY

Document	Date of Issue	Overall Rationale
V160-002-02	07-May-2019	Updated cCMVi Case Definition, clarified the infant sample collection strategy, and adjusted visit schedules to be more accommodating and maintain more contact with study participants
V160-002-01	06-Aug-2018	Updated protocol procedures for clarification
V160-002-00	31-Oct-2017	Original Protocol

PROTOCOL AMENDMENT SUMMARY OF CHANGES

Amendment [02]

Overall Rationale for the Amendment:

Updated the cCMVi Case Definition, clarified the infant sample collection strategy and adjusted visit schedules to be more accommodating and maintain more contact with study participants

Summary of Changes Table:

Section # and Name	Description of Change	Brief Rationale
2.1 Schedule of Activities: Screening Through Treatment Phase	Changed Visit 3/Month 2 visit window to ± 21 days; Changed Visit 4/Month 6 visit window to ± 28 days.	The visit windows for the vaccination visits at Month 2 and Month 6 were widened to allow more flexibility with scheduling these visits.
2.2 Schedule of Activities: Follow-up Period	Changed Month 18 telephone contact to Study Visit; Added Month 30 Study Visit.	Two in-clinic study visits are being added to maintain participant-site contact and promote retention of study participants. The additional visits will also allow for more efficient management of urine/saliva self-collection kits. One visit/row was added to SoA 2.2, and the other to SoA 2.3.

Section # and Name	Description of Change	Brief Rationale
2.3 Schedule of Activities for Congenital Cytomegalovirus Infection Assessment	• Clarified the visit window for cCMVi Visit 2. • Added a row for an activity to record any anti-CMV treatments received by participants during pregnancy. • Added a column for "Additional cCMVi visit (if necessary)" • Added a Notes column and moved saliva collection information to column	• cCMVi Visit 2 should be scheduled between the day of birth and up to 14 days after birth. A window of +7 days is specified to ensure infant samples are collected within 21 days of birth (a requirement of the cCMVi case definition). • Collection of anti-CMV treatments received by participants during pregnancy is needed for comprehensive documentation of pregnancy outcomes. • The optional additional visit was added to allow for the collection of a 2nd set of infant urine and saliva samples, if they need to be collected on a separate day. The visit window on this visit is also specified to ensure that the 2nd set of infant samples is collected within 21 days of birth (a requirement of the cCMVi case definition) • Clarification of information
5.1.1 Study Diagram	Updated the Study Diagram to include on−site visits at Months 18 and 30.	The updates were added for consistency with the Schedule of Activities (SoA).
6.2.1 Participant Deferment Criteria before Vaccination	Deleted language stating the participant would only be permitted to continue with a delayed vaccination if the vaccine can be administered within the allowed window	The requirement to only allow delayed vaccination if the vaccine can be administered within the allowed window is unnecessarily restrictive.

Section # and Name	Description of Change	Brief Rationale
7.3 Method of Treatment Assignment	Clarified that approximately 360 participants (or as many as operationally feasible) will be included in the Detailed Immunogenicity sub-study	Due to the high screen failure rate, it may not be logistically feasible to enroll 360 participants in the Detailed Immunogenicity Sub-Study as initially planned for this exploratory endpoint.
8.1 Discontinuation of Study Treatment	• Clarified that the self-collection of urine and saliva samples by study participants should continue regardless of whether they complete the entire vaccine regimen • Removed last bullet point stating that a participant who has received any prohibited medications or vaccinations since Dose 1 administration must be discontinued and added a bullet for "Received a non-study CMV vaccine."	• Statement added to emphasize that self-collection of urine and saliva samples by study participants should continue regardless of whether they complete the entire vaccine regimen. • The requirement for participants to be discontinued from study treatment if they received any prohibited medications or vaccination since Dose 1 is unnecessarily restrictive. However, participants who receive a non-study CMV vaccine must be discontinued from study treatment.
Section 9 Study Assessments and Procedures	Updated visit numbers for the Month 24 and 36 sample collections in Table 3.	Visit numbers in Table 3 were updated for consistency with the new visit numbers for the Month 24 and Month 30 visits in the SoA.
9.1.8 Treatment Administration	Updated first sentence to expand information regarding qualified study personnel	Conformation with current template language

Section # and Name	Description of Change	Brief Rationale
9.2.1.1.2 CMVi Case Detection	Clarified that the monthly urine and saliva samples may be collected outside of the study site or at scheduled site visits (when applicable), as indicated in the SoA	Urine and saliva samples are self-collected by the participant at monthly intervals. For intervals that coincide with scheduled study visits, it is permissible for the samples to be collected during the on-site visit.
9.2.1.2.1 cCMVi Case Definition	Updated cCMVi Case Definition	With input from external experts and regulatory agencies, it has been determined that only cases of cCMVi which have been virologically confirmed will be consider as endpoints for analysis. For this reason, "Pregnancy impacted by CMV but CMV not virologically confirmed" has been removed from the cCMVi case definition. Language was also revised to provide further clarification.
9.2.1.2.2 cCMVi Case Detection	Updated cCMVi case detection procedures • Clarified that collection of anti-CMV treatments received by participants during pregnancy will be collected for the duration of the study. Added statement that "all relevant medical records should be placed in the participant's trial file." • Clarified that 2 sets of urine and saliva will be collected from the infant within 21 days of birth. • Revised the trigger for adjudication to be suspicion of congenital CMV during pregnancy of a study participant or in the infant.	• Collection of anti-CMV treatments received by participants during pregnancy is needed for comprehensive documentation of pregnancy outcomes. Clarification of information. • 2 sets of infant urine and saliva samples collected within 21 days of birth are needed to support the updated cCMVi Case Definition. • Because the cCMVi Case Definition was revised, the trigger for adjudication was revised accordingly.

Section # and Name	Description of Change	Brief Rationale
9.3.1 Time Period and Frequency for Collecting AE, SAE and Other Reportable Safety Event Information	In Table 4, in the row for "Lactation Exposure" added the following information: • Reporting Time Period: After the Protocol Specified Follow-up Period 2 column: "Previously reported – follow to completion". • Timeframe to Report Event and Follow-up Information to SPONSOR column: "Within 24 hours of learning of event."	In V160-002-01, the "Reporting Time Period: After the Protocol Specified Follow-up Period 2" and "Timeframe to Report Event and Follow-up Information to SPONSOR" columns were inadvertently left blank in the Lactation Exposure row.
9.3.3 Follow-up of AE, SAE and Other Reportable Safety Event Information	Clarified that participants who inadvertently become pregnant before receiving all 3 doses of study vaccine or placebo, and any participant who becomes pregnant after the treatment phase will remain in the trial for follow-up of pregnancy and infant outcome	Previous text was ambiguous and clarifies that all pregnancies will be followed for outcome regardless of the vaccination status of the participant.
10.5.1 Efficacy Analysis Populations	Changed PPE Analysis population definition for all arms to include all 3 doses	The definition was revised to minimize potential bias in efficacy analyses.

Section # and Name	Description of Change	Brief Rationale
12.3 Appendix 3 Contraceptive Guidance and Pregnancy Testing	Removed bilateral tubal ligation occlusion as allowable contraceptive. Added statement: "As WOCBP are the target population of this study and pregnancy is permissible after the treatment phase, permanent contraception methods at study entry are not allowed."	This study is designed to enroll women of childbearing potential (WOCBP). Bilateral tubal ligation occlusion is a permanent procedure therefore it is not an allowable contraceptive method.
12.4 Appendix 4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting	Updated text in Assessment of Intensity section.	Conformation with current template language
Additional non-substantive editorial/grammatical/typographical changes were made as appropriate.		

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1. Synopsis

Protocol Title:

Double-Blind, Randomized, Placebo-Controlled Phase 2b, Multi-center Study to Evaluate the Safety, Tolerability, Efficacy and Immunogenicity of a 2-Dose and a 3-Dose Regimen of V160 (Cytomegalovirus [CMV] Vaccine) in Healthy Seronegative Women, 16 to 35 Years of Age

Short Title:

V160 2-Dose and 3-Dose Regimens vs. Placebo in Healthy CMV Seronegative Females

Objectives/Hypotheses and Endpoints:

In healthy females between 16 and 35 years of age:

Objective/Hypothesis	Endpoint
Primary	
• **Objective**: To demonstrate the efficacy of a 3-dose regimen of V160 in reducing the incidence of primary CMVi during the follow-up period after the last dose of the vaccine **Hypothesis 1**: Administration of a 3-dose regimen of V160 will reduce the incidence of primary CMVi compared to placebo. (*The statistical criterion for success requires the lower limit of the 95% confidence interval [CI] of vaccine efficacy [VE] to be greater than 0%*).	• Incidence of CMVi in the 3-dose vaccine and placebo groups during the follow-up period after the last dose of a 3-dose regimen of the vaccine
• **Objective**: To assess the safety and tolerability of a 2-dose and a 3-dose regimen of V160	• Number of participants in the 2-dose and 3-dose vaccine groups experiencing solicited injection site adverse events (AEs) within Days 1 through 5 after each vaccination visit; solicited systemic AEs; and vaccine-related serious AEs (SAEs) within Days 1 through 14 after each vaccination visit

Secondary	
• **Objective**: To demonstrate the efficacy of a 2-dose regimen of V160 in reducing the incidence of primary CMVi during the follow-up period after the last dose of the vaccine **Hypothesis 2**: Administration of a 2-dose regimen of V160 will reduce the incidence of primary CMVi compared to placebo. (*The statistical criterion for success requires the lower limit of the 95% CI of VE to be greater than 0%*).	• Incidence of CMVi in the 2-dose vaccine and placebo groups during the follow-up period after the last dose of a 2-dose regimen of the vaccine

Overall Design:

Study Phase	Phase 2b
Clinical Indication	Prevention of CMVi
Population	Participants will be healthy, CMV seronegative, non-pregnant, female, age 16-35 years Infants of participants will be followed for congenital CMVi
Study Type	Interventional
Type of Design	Parallel, controlled, multi-center
Type of Control	Placebo
Study Blinding	Double-blind
Estimated Duration of Trial	The Sponsor estimates that the trial will require approximately 3 years from the time the first participant signs the informed consent/assent until the last participant's last study-related phone call or visit. For purposes of analysis and reporting, the overall study ends when the Sponsor receives the last laboratory result or at the time of final contact with the last participant, whichever comes last.

Number of Participants:

Approximately 2100 participants will be enrolled.

Treatment Groups and Duration:

Treatment Groups	3 treatment groups, 1:1:1 randomization as follows: • Active treatment group will receive 3 doses of vaccine V160 (100 Units/0.5 mL dose with Merck aluminum phosphate adjuvant [MAPA], 4°C stable formulation) administered intramuscularly (IM) according to a Day 1, Month 2, Month 6 schedule • Active treatment group will receive 2 doses of vaccine V160 (100 Units/0.5 mL dose with MAPA, 4°C stable formulation) administered IM according to a Day 1 (V160), Month 2 (placebo-saline solution), Month 6 (V160) schedule • A control group will receive 3 doses of placebo (saline solution) administered IM according to a Day 1, Month 2, Month 6 schedule
Duration of Participation	A participant may be in the trial for up to approximately 3 years, depending on when they are enrolled. Because this is an endpoint driven study, participation times will vary depending on when the participant is enrolled (subjects will be enrolled over an 18- month period and the study is planned to be a 3-year trial overall, based on reaching the primary endpoint).

Study governance considerations are outlined in Appendix 1. A list of abbreviations used in this document can be found in Appendix 6.

2. Schedule of Activities (SoA)

2.1 Schedule of Activities: Screening Through Treatment Phase

Trial Period	**Screening**						
Visit Number/Title	**Visit 1 Screening**	**Visit 2 Day 1 Vacc 1**	**Visit 3 Month 2 Vacc 2**	**Visit 4 Month 6 Vacc 3**	**Visit 5 Month 7**	**Reminder Contacts (Monthly)**	**Notes**
Scheduled Day	**-21 to -1**	**1**	**61**	**183**	**213**		
Scheduling Window	-21 to -1	Day 1	± 21 days	±28 days	21 to 49 days after Vacc 3		
Informed consent	X						
Informed consent for future biomedical research	X						
Check inclusion/exclusion criteria	X	X					
Medical history	X						
Prior/concomitant medication review	X	X	X	X	X		
Full physical examination		X					Includes height, weight, vital signs (temperature, heart rate, respiratory rate, and blood pressure), and other assessments specified in Section 9.5.1
Screening for CMV serostatus by IgG	X						
Serum pregnancy test (β-HCG)	X						
Participant identification card	X						Card provided after informed consent. Site to add randomization number at Visit 2
Treatment randomization		X					
Urine and saliva sample self-collection training		X					Will be performed prior to vaccination
Directed physical examination			X	X	X		Includes vital signs (temperature, heart rate, respiratory rate, and blood pressure), and limited assessments specified in Section 9.5.1
Urine pregnancy test		X	X	X	X		Will be performed prior to vaccination

Trial Period	**Screening**						
Visit Number/Title	**Visit 1 Screening**	**Visit 2 Day 1 Vacc 1**	**Visit 3 Month 2 Vacc 2**	**Visit 4 Month 6 Vacc 3**	**Visit 5 Month 7**	**Reminder Contacts (Monthly)**	**Notes**
Scheduled Day	**-21 to -1**	**1**	**61**	**183**	**213**		
Scheduling Window	-21 to -1	Day 1	± 21 days	±28 days	21 to 49 days after Vacc 3		
Participants NOT included in the Detailed Immunogenicity sub-study: Serum for NAbs and IgG ELISA antibodies		X			X		Baseline sample to be collected prior to vaccination
Detailed Immunogenicity sub-study participants only: Blood for CMV IFN-γ ELISPOT (PBMCs) and serum for NAbs and IgG ELISA antibodies		X		X	X		Baseline and Month 6 samples to be collected prior to vaccination
Blood (DNA) for future biomedical research		X					Will be performed prior to vaccination
V160/Placebo administration		X	X	X			
30-minute postdose safety observation		X	X	X			
Distribution of eVRC device		X					
Check postdose 1 exclusion criteria (and any contraindication to vaccination)			X	X			Will be performed prior to vaccination

Trial Period	**Screening**						
Visit Number/Title	**Visit 1 Screening**	**Visit 2 Day 1 Vacc 1**	**Visit 3 Month 2 Vacc 2**	**Visit 4 Month 6 Vacc 3**	**Visit 5 Month 7**	**Reminder Contacts (Monthly)**	**Notes**
Scheduled Day	**-21 to -1**	**1**	**61**	**183**	**213**		
Scheduling Window	-21 to -1	Day 1	± 21 days	±28 days	21 to 49 days after Vacc 3		
Adverse event monitoring	X	X	X	X	X		Screening to randomization: All AEs, SAEs, and other reportable events that cause the participant to be excluded from the trial or are the result of a protocol-specified intervention are to be reported. Randomization through 14 days following Dose 3: AEs (serious and non-serious) are to be reported from Days 1 through 14 following each vaccination. SAEs, deaths, other reportable events (ie, cancer, overdose, pregnancy, and infant SAEs) are to be reported throughout the duration of an individual's study participation. Exposure during breastfeeding is to be reported from Day 1 through Month 7.
Review of eVRC data			X	X	X		
Reminder contact for urine/saliva self-collection and pregnancy status						X	
Urine and saliva for CMV		X	Collected monthly				Self-collected by participant starting at Day 1 (prior to vaccination) and then continue monthly through ≈Month 36 or beyond, depending on event accrual

Vacc = Vaccine; eVRC = Electronic vaccination report card; NAbs = neutralizing antibodies; β-hCG = Human chorionic gonadotropin; IgG = immunoglobin G; IFN-γ = Interferon gamma; ELISA = Enzyme-linked immunosorbent assay; PBMC = peripheral blood mononuclear cell; DNA = Deoxyribonucleic acid; CMV = Cytomegalovirus; AE = Adverse event; SAE = Serious adverse event

2.2 Schedule of Activities: Follow-up Period Through Approximately Month 36 (Approximate Time Point to Reach Cases Required for Primary Endpoint Assessment)

Trial period	**Follow-up period**						**Notes**
Visit number/title	**Visit 6 Month 12 Year 1**	**Visit 7 Month 18 Year 1.5**	**Visit 8 Month 24 Year 2**	**Visit 9 Month 30 Year 2.5**	**Visit 10 Month 36 Year 3**	**Reminder contacts (monthly)**	Study closeout activities will be conducted at the participant's closest visit to the end of the study. Close out activities must be completed in the event of early discontinuation. Since the study completion is driven by the primary endpoint (i.e., number of events), the number of visits will depend on the timing of event accrual.
Scheduled Day	**365**	**548**	**731**	**914**	**1096**		
Scheduling Window	± 28 days	± 28 days	± 28 days	± 28 days	± 90 days		
Reminder contact for urine/saliva self-collection and pregnancy status						X	
Deaths and SAEs monitoring	X	X	X	X	X		
Participants NOT included in the Detailed Immunogenicity sub-study: Serum for NAbs and IgG ELISA antibodies	X		X		X		
Detailed Immunogenicity sub-study participants only: Serum for NAbs and IgG ELISA antibodies Blood for CMV IFN-γ ELISPOT (PBMCs)	X		X		X		
Urine and saliva sample for CMV	collected monthly						Self-collected by participant starting at Day 1 (prior to vaccination) and then continue monthly through ≈Month 36 or beyond, depending on event accrual

SAE = serious adverse event; NAbs = neutralizing antibodies; IgG = immunoglobin G; ELISA = Enzyme-linked immunosorbent assay; ELISPOT = Enzyme-linked immunosorbent spot; PBMC = peripheral blood mononuclear cell; IFN-γ = Interferon gamma

2.3 Schedule of Activities for Congenital Cytomegalovirus Infection Assessment

Trial period visit number/title	**cCMVi visit 1**	**Monthly pregnancy contacts**	**cCMVi visit 2**	**Additional cCMVi visit (if necessary)**	**Reminder contacts (monthly)**	**cCMVi infant follow-up, yearly contacts for 3 years**	**Notes**
Scheduled day	Pregnancy reported	Once per month	Up to 14 days after birth of the infant	Up to 14 days after birth of the infant			
Scheduling window	-	-	+ 7 days	+ 7 days		-	
Reminder contact for urine/saliva self-collection and pregnancy status					X		
Provide instruction on pregnancy and infant follow-up	X						
Contact from site to assess progress of pregnancy including any suspected congenital CMV infection		X					
Record anti-CMV treatments received by participants during pregnancy		X					
Pregnancy/infant outcome assessment		X	X	X			
Informed consent for infant urine and saliva sample if live birth	X						
Infant urine and saliva sample collected for CMV PCR			X	X			Saliva sample should not be collected within 2 hours of breastfeeding. Two sets of samples (saliva and urine) must be collected at 2 different time points separated by at least 3 hours within 14 (+7) days after birth
SAE monitoring and collection for live birth infants		X	X	X		X	
Yearly follow-up for hearing, vision, and other neurologic outcomes (for infants with cCMVi only)						X	

cCMVi = congenital cytomegalovirus infection; PCR = Polymerase chain reaction

3. Introduction

3.1 Study Rationale

Merck is developing an investigational CMV vaccine (referred to as V160) for the prevention of infection and disease caused by CMV in vulnerable populations. Although CMVi is asymptomatic in healthy individuals, CMV is an important pathogen for congenitally infected infants and immunosuppressed individuals, such as solid organ transplant or hematopoietic stem cell transplant recipients.

Congenital cytomegalovirus infection (cCMVi) is the most frequent infectious cause of newborn malformation in developed countries. Congenital CMVi, which can lead to congenital CMV disease (cCMVd), presents as sensorineural hearing loss (responsible for almost one-third of all cases), and neurodevelopmental abnormalities. As many affected children require significant ongoing care and special therapeutic and educational services, the economic burden associated with congenital CMV infection is substantial. In the United States (US) in 2000, the estimated annual cost of congenital CMV infection is up to $2 billion in 1992 dollars, which correlates with in excess of $3 billion in 2017 [Marsico, C. 2017]. In a study of economic burden in Germany in 2011, the total cost of CMV infection in Germany per child and for the society as a whole, amounted to 242.91 million euros (940.77 million euros, undiscounted) annually [Walter, E,, et al 2011].

The impact of cCMVd on public health is likely significantly under-appreciated with very low public awareness, largely because relatively few infected infants are severely ill at birth and symptoms may not be recognized in the newborn. When the later symptoms of cCMVd, such as visual and hearing impairment, do appear, the diagnosis of CMV can no longer be confirmed. Congenital CMVd occurs more frequently than Down's syndrome, Spina Bifida, Fetal Alcohol Syndrome and, prior to the availability of a vaccine, non-epidemic Congenital Rubella Syndrome. Development of a vaccine for prevention of cCMVi has been rated a high priority by the National Academy of Medicine [Griffiths, P. D., et al 2011]. Refer to Section 3.2 for more details regarding burden of disease.

The V160 development strategy focuses initially on women of childbearing age. The vaccine was developed based on the principle that an effective CMV vaccine should be designed to parallel the immune response commonly seen in healthy CMV seropositive participants and should induce both humoral and cellular immunity.

3.2 Background

Disease

Human CMV, also known as human herpesvirus 5 (HHV-5) is a double-stranded deoxyribonucleic acid (DNA) virus belonging to the beta-herpesvirus family of *Herpesviridae*. Acquisition appears to require close or intimate contact with persons who are excreting CMV in their urine, saliva or other secretions. CMV can also be transmitted via blood transfusion, breast milk, sexual intercourse, and transplanted organs.

In most healthy individuals, CMVi is asymptomatic. When symptoms are present, they are often mild, can be confused with other illnesses, and include fever, sore throat, fatigue, and/or swollen glands. After infection, the virus establishes lifelong latency, the evidence of

which is the detection of anti-CMV antibodies in the blood (CMV seropositivity). Healthy individuals with latent CMV infection can reactivate to shed the virus in their saliva or urine, which is also predominantly asymptomatic.

It is known that CMV can cause serious disease in congenitally infected newborns and immunocompromised individuals. The spectrum of disease in newborns with cCMVi ranges from fetal/infant death to neurological and sensory impairments which are diagnosed later in childhood [Hahn, G., et al 1998] [Just-Nubling, G., et al 2003] [Sinclair, J. 2008] [Lanzieri, T. M., et al 2014].

Diagnosis

Incident CMVi can be diagnosed by finding new anti-CMV antibodies in the blood (in a previously CMV seronegative individual) or by detection of CMV DNA by real time quantitative polymerase chain reaction (PCR) in the blood, saliva, or urine.

In immunocompromised individuals, CMV PCR in the blood is the diagnostic method of choice. In newborns and immunocompetent adults, detection of CMV DNA in the saliva and urine is the preferred method of diagnosis. After a new CMVi, CMV DNA can be detected in the saliva or urine for 6-8 weeks in adults (and perhaps longer). Latently CMV infected individuals (CMV seropositive subjects) can also have intermittent reactivation evidenced by detection of CMV DNA in saliva and urine; the triggers of this “viral shedding” are unknown.

The focus of this study will be CMV detection in saliva and urine. Refer to Section 5.4.1.1 regarding the rationale for the CMVi endpoint.

Epidemiology and Disease Burden

In developed countries, maternal seropositivity/seroprevalence (IgG positivity) for CMV is approximately 50% and the prevalence of cCMVi among infants is estimated to be approximately 0.7% among live births. In developing countries, maternal seroprevalence is usually higher than in developed countries, and ranges from 84 to 100%. The prevalence of cCMVi in developing countries varies substantially, both within and between countries, and has been reported to range from 0.6% to 6.1% [Dollard, S. C., et al 2007] [Kenneson, A. and Cannon, M. J. 2007] [Lanzieri, T. M., et al 2014] [Wang, S., et al 2017]. The rate of CMVi during pregnancy is assumed to be similar to the rate in the general population (approximately 1% to 4% depending on risk factors), although data are limited.

The CMV seropositivity rate in a population (a surrogate marker for CMVi) is affected by several factors. CMV seroprevalence varies by age (increase with age), race/ethnicity, and region. African Americans and Hispanics are infected with CMV at a younger age than non-Hispanic white individuals in the US, and approximately 90% are CMV seropositive by 40 years of age. Low socioeconomic status, residence in an urban area, and a history of sexually transmitted diseases are also risk factors for CMV seropositivity [Bate, S. L., et al 2010]. In studies conducted in Europe, CMV seroprevalence was similar to the US and was highest among non-Western, immigrant groups.

In the 1999 to 2004 NHANES survey of US general population, the annualized rates of CMV seroconversion were about 1.6% in the US overall, and were 5.7%, 5.1% and 1.4% among black, Mexican-American, and non-Hispanic white participants aged 12-49 years,

respectively [Colugnati, F. A., et al 2007]. The strongest risk factor for CMV seroconversion is exposure to young children, particularly those attending daycare [Hyde, T. B., et al 2010].

It is estimated that of the approximately 4 million live births each year in the US, approximately 0.5%-2.0% (20,000-80,000) will have cCMVi [Dollard, S. C., et al 2007] [Sung, H. and Schleiss, M. R. 2010]. In other developed countries (in Europe, Japan), rates have been reported to be similar, 0.65 per 100 births. At least 15% (3,000 to 9,000 in the US) will develop symptoms of CNS damage either at birth or progressively in the first few years of life, with clinical manifestations including sensorineural hearing loss, mental retardation, and cerebral palsy.

Refer to the investigator's brochure (IB) for additional details.

3.2.1 Pharmaceutical and Therapeutic Background

There is no effective CMV vaccine to protect women from acquiring CMVi during pregnancy or any recognized intervention that can effectively reduce transmission of CMV from newly infected pregnant women to the fetus.

Immune responses to natural CMVi can protect individuals against re-infection [Plotkin, S. A., et al 1989] [Adler, S. P., et al 1995] [Wang, D., et al 2016]. Maternal immunity from naturally acquired infection can protect a fetus from cCMVi [Fowler, K. B., et al 2003]. In addition, CMV-seropositivity is associated with a lower incidence of severe CMV disease post-transplantation [Opelz, G., et al 2004]. An effective CMV vaccine should therefore be designed to parallel the immune response commonly seen in healthy CMV seropositive individuals and should induce both humoral and cellular immunity. Such a vaccine could protect women from acquisition of CMVi, and thereby prevent vertical transmission to their baby, and would be a major medical advance that addresses a critical unmet public health need.

3.2.2 Pre-clinical and Clinical Studies

3.2.2.1 Pre-Clinical

The AD169 strain of CMV was originally isolated at the National Institutes of Health from the adenoids of a 7-year-old girl. The isolate was attenuated through multiple passages in human fibroblasts. This live attenuated virus was tested as a vaccine in a Phase-1 study in 24 CMV seronegative male participants by Neff, et.al., [Weibel, R. E., et al 1980]. The vaccine was well tolerated and did not cause any SAEs. No virus could be recovered either from peripheral lymphocytes or urine of the vaccinated participants.

The V160 vaccine was created using the Merck variant of AD169 virus (MAD169). The vaccine virus was modified to be conditionally replication defective in the absence of a synthetic molecule called Shld-1 through the ddFKBP-mediated destabilization of two viral proteins critical for viral replication. With Shld-1 present, viral stocks for V160 can be grown, but in vivo with no Shld-1, replication of V160 is prevented. In order to facilitate broad immune responses, the pentameric complex (gH/gL/pUL128-131) was restored on the V160 virus. This restores elicitation of antibodies against the gH antigens, as well as viral tropism of V160 for epithelial and endothelial cells, permitting entry and some protein

production without replication (in the absence of Shld-1). Cell-mediated and humoral antibody responses are thereby enhanced.

Since there is no relevant animal model available to assess CMV vaccine efficacy (VE), the key immunological parameters for preclinical evaluations were based on neutralizing antibodies (NAbs) and T-cell responses in animals. V160 has been extensively evaluated in rhesus macaques and can elicit potent neutralizing antibodies superior to those seen with the gB/MF59 candidate CMV vaccine as well as balanced CD4 and CD8 T cell responses. V160 was well tolerated in toxicology studies in animals supporting the administration of this vaccine to humans.

Refer to the IB for further details regarding viral replication, virological characteristics of the vaccine virus, as well as detailed background information on animal studies.

3.2.2.2 Clinical Studies

Phase 1 Clinical Study

The first-in-human study was a Phase 1 dose ranging study to assess the safety and immunogenicity of various antigen dose levels, formulations, and routes of administration in healthy volunteers 18 years of age and older. In this study, the safety and tolerability as well as the immunogenicity of V160 was evaluated in both CMV seronegative and seropositive participants. The Phase 1 study is briefly summarized below. Refer to the IB for more detailed information.

Participants in the Phase 1 study received a 3-dose vaccination regimen (0-1-6-month schedule) at various doses (10-250 units) administered intramuscularly or intradermally, with and without MAPA adjuvant. A saline placebo was used as the control.

In initially CMV seronegative participants, all vaccination groups receiving V160 with and without MAPA exhibited statistically significant higher CMV-specific neutralizing antibody titers compared with the placebo group. The neutralizing antibody geometric mean titer (GMT) at Month 7 was highest in recipients of V160 100-unit dose + MAPA. Robust cell-mediated immune (CMI) responses were also noted among CMV seronegative participants but no differences across dose groups of V160 were observed. Overall, CMI responses at all doses, and neutralizing antibody responses at some doses (including 100U+MAPA) in CMV seronegative participants approximated those observed in CMV seropositive participants.

All tested V160 formulations were safe and generally well-tolerated. No SAEs and no events of clinical interest were reported. Overall, adjuvant-containing formulations were more reactogenic than unadjuvanted irrespective of dose. Across the different vaccination groups, the majority of AEs were the solicited injection site AEs (pain/tenderness, erythema/redness, and swelling) and the solicited systemic AEs (headache, fatigue, muscle pain, and joint pain); all of these events were transient, and most were mild to moderate in intensity. No vaccine-strain CMV was detected by PCR in the saliva or urine of vaccine recipients.

Phase 2 clinical study

The Phase 2b study protocol described herein will be conducted to further evaluate the V160 formulation containing 100 units/dose + MAPA, which elicited high CMV-specific neutralizing antibody in the Phase 1 study. Both humoral and cellular immune responses as well as protection against CMVi in vaccine recipients will be assessed.

The targeted population in this trial is women of child-bearing age and thus, healthy females between 16 and 35 years of age will be included in the study. Infant outcomes of any study participant who becomes pregnant will be assessed to evaluate protection against cCMVi as well.

Refer to Section 5.4 for the scientific rationale for the study endpoints and use of placebo and to Section 5.5 for dose justification.

3.2.2.3 V160 Replication

As described, the V160 vaccine virus is built on a live-attenuated virus backbone with a designed block of V160 replication by a chemical genetic switch. Because of this novel design, V160 is a conditionally replication defective virus and its replication requires the presence of Shld-1.

There is a theoretical possibility that certain medications taken by participants could mimic Shld-1 to stabilize ddFKBP and achieve sufficient plasma concentrations to inadvertently sustain replication in vivo, at local injection sites. Based on results of a cell-based assay with high-through-put capacity for screening of medicinal agents that can mimic Shld-1, only tacrolimus was found to be able to stabilize ddFKBP. As a precaution, participants taking tacrolimus will be excluded. Refer to the IB for details.

In this Phase 2b study, urine and saliva samples will be collected and analyzed for CMV by PCR during the vaccination period and thereafter through Month 36. Any detected virus will be typed to distinguish non-vaccine strain CMV (causing a case of CMVi) from vaccine strain. Refer to Section 5.4.1.1 for additional details.

3.3 Benefit/Risk Assessment

Congenital CMVi can lead to life-long sequelae in the infected newborns. A focus on prevention of primary CMVi in women of childbearing age is therefore needed. V160 has demonstrated an acceptable safety profile, with immunogenicity that approaches natural infection with CMV (known to be protective against cCMVi). These results warrant further evaluation of the safety, immunogenicity, and efficacy of this investigational vaccine.

It cannot be guaranteed that participants in clinical trials will directly benefit from treatment during participation, as clinical vaccine trials are generally designed to provide information about the safety, immunogenicity, and/or efficacy of an investigational vaccine.

Additional information regarding specific benefits and risks for participants in this clinical trial may be found in the accompanying IB and informed consent documents.

4. Objectives/Hypotheses and Endpoints

In healthy females between 16 and 35 years of age:

Objective/Hypothesis	Endpoint
Primary	
• **Objective**: To demonstrate the efficacy of a 3-dose regimen of V160 in reducing the incidence of primary CMVi during the follow-up period after the last dose of the vaccine • **Hypothesis 1**: Administration of a 3-dose regimen of V160 will reduce the incidence of primary CMVi compared to placebo. (*The statistical criterion for success requires the lower limit of the 95% CI of VE to be greater than 0%*)	• Incidence of CMVi in the 3-dose vaccine and placebo groups during the follow-up period after the last dose of a 3-dose regimen of the vaccine
• **Objective**: To assess the safety and tolerability of a 2-dose and a 3-dose regimen of V160	• Number of participants in the 2-dose and 3-dose vaccine groups experiencing solicited injection site AEs within Days 1 through 5 after each vaccination visit; solicited systemic AEs; and vaccine-related SAEs within Days 1 through 14 after each vaccination visit
Secondary	
• **Objective**: To demonstrate the efficacy of a 2-dose regimen of V160 in reducing the incidence of primary CMVi during the follow-up period after the last dose of the vaccine • **Hypothesis 2**: Administration of a 2-dose regimen of V160 will reduce the incidence of primary CMVi compared to placebo. (*The statistical criterion for success requires the lower limit of the 95% CI of VE to be greater than 0%*).	• Incidence of CMVi in the 2-dose vaccine and placebo groups during the follow-up period after the last dose of a 2-dose regimen of the vaccine

Objective/Hypothesis	Endpoint
Exploratory	
• To assess and compare the kinetics of humoral immune response induced by 2-dose and 3-dose regimens of V160	• Percent of participants who seroconvert based on the NAb and IgG ELISA assays from CMV seronegative at Day 1 to CMV seropositive at Month 7[a] • Seropositivity based on the NAb and IgG ELISA assays at Day 1, Months 6, 7[a], 12, 24, and 36 • GMT based on the NAb and the IgG ELISA assays at Day 1, Months 6, 7[a], 12, 24, and 36
• To assess and compare the kinetics of CMI response induced by 2-dose and 3-dose regimens of V160 in a subset of study participants[b]	• Geometric mean count (GMc) of spot forming cells (SFC) per million PBMCs (SFC/10^6 PBMCs) based on the ELISPOT assay at Day 1, Months 6, 7[a], 12, 24 and 36
• To evaluate the incidence of cCMVi in infants born to recipients of 2-dose and 3-dose regimens of V160 compared to placebo, during the follow-up period after the last dose of vaccine or placebo	• Incidence of cCMVi cases in the V160 2-dose, V160 3-dose, and placebo groups during the follow-up period after the last dose of vaccine or placebo
• To follow the outcomes of infants diagnosed with cCMVi in the study	• Hearing, vision, and neurological outcomes of infants diagnosed with cCMVi over 3 years
• To explore potential correlations between humoral and CMI response to 2-dose and 3-dose regimens of V160 and efficacy of 2-dose and 3-dose regimens of V160 against CMVi	• Immune responses in participants in the 2-dose and 3-dose vaccine groups with and without CMVi during the follow-up period after the last dose of V160

[a] Month 7 is the time point corresponding to 1 month following last dose of the 2-dose and 3-dose regimen of V160
[b] In Detailed Immunogenicity sub-study participants only (N≈360).
cCMVi = Congenital CMV infection; CMV = Cytomegalovirus; CMVi = CMV infection;
DNA = Deoxyribonucleic acid; ELISA = Enzyme-linked immunosorbent assay
ELISPOT = Enzyme-linked immunosorbent spot; IgG = Immunoglobulin G
NAb = Neutralizing antibody
PCR = Polymerase chain reaction; PBMC = Peripheral blood mononuclear cells
SFC = Spot forming cells

5. Study Design

5.1 Overall Design

This study is a randomized, placebo-controlled, parallel-group, multi-center, double-blind trial of V160, in healthy CMV seronegative adolescent and adult women 16 to 35 years of age. Refer to Section 6 for details regarding the study population.

This is a proof-of-concept study intended to evaluate the safety and efficacy against CMVi of a 3-dose and 2-dose regimen of V160 administered over 6 months compared to placebo. The time period for the treatment phase of the study is Day 1 through Month 7. The study is event-driven, requiring a fixed number of CMVi cases to be accumulated in order to test the hypotheses relating to the efficacy of 3-dose and 2-dose regimens of V160. The study is designed to accumulate the required number of CMVi cases in ≈3 years with ≈2100 study participants. Since the study duration is driven by the primary endpoint (ie, number of events), the study duration will depend on the timing of event accrual. The study will also evaluate the immunogenicity of a 3-dose and 2-dose regimen of V160 (humoral immunity in the whole study and detailed immunogenicity including CMI in an approximately 360-participant sub-study) as well as the impact of administration of V160 on the incidence of cCMVi in participants who get pregnant during the study.

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Trial SoA - Section 2. Details of each procedure are provided in Section 9 – Study Assessments and Procedures.

There will be 1 formal interim analysis (IA) for testing the primary hypothesis addressing the efficacy of a 3-dose regimen of V160 compared to placebo, to be conducted upon accumulation of 15 total cases of CMVi in the combined V160 3-dose and placebo arms of the study. The interim analysis will also assess futility. It is anticipated that the required 15 cases will be accumulated at ≈18 months after enrollment of the first study participant.

An external data monitoring committee (eDMC) will be formed to conduct an interim analysis of the efficacy data, and a review of the safety data, collected during this study. Results of the IA will be reviewed by the eDMC, who will make recommendations to the Sponsor’s Executive Oversight Committee. Additional details regarding the responsibilities of the eDMC will be provided in an eDMC charter.

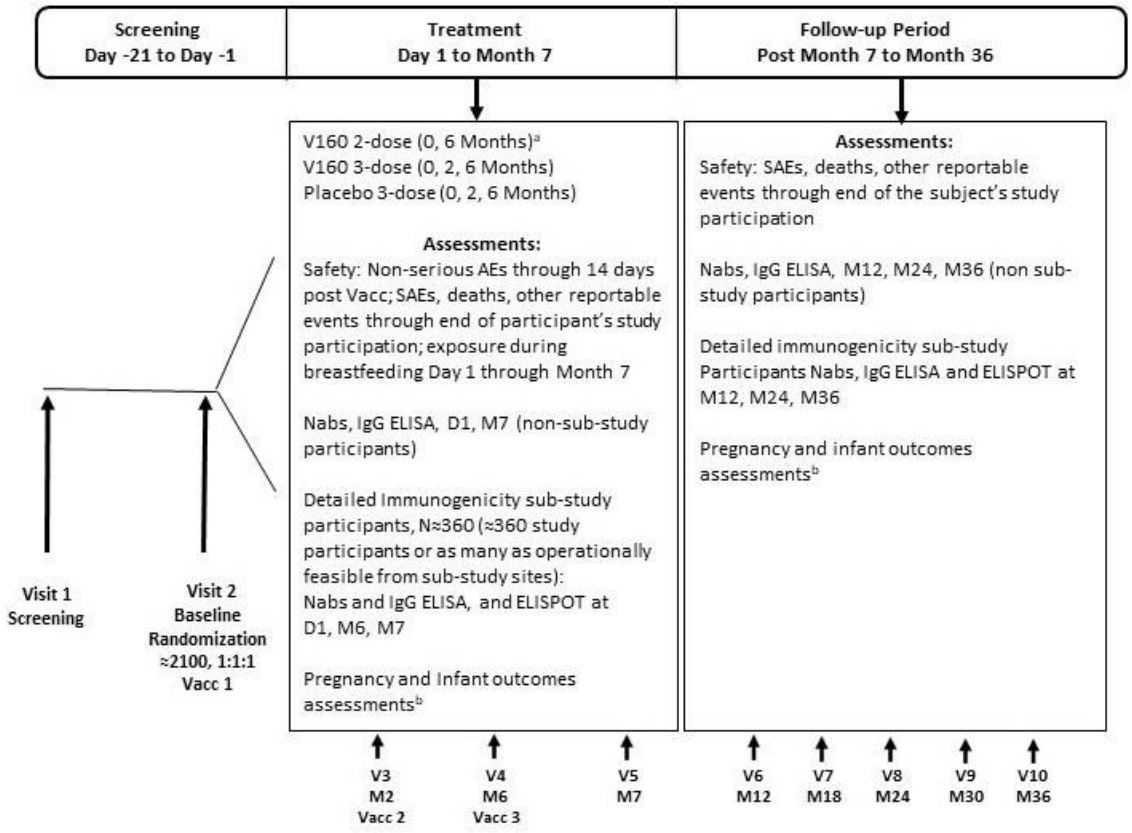
Details relating to the planned IA are provided in Section 10.7. There will be no IA for the testing of the secondary hypothesis addressing the efficacy of a 2-dose regimen of V160 compared to placebo.

All pregnancies that occur during the study will be monitored for evidence of cCMVi in the fetus/infant. Determinations of cCMVi will be conducted by an independent, blinded adjudication committee. Refer to Section 9.2.1.2.2 for further details.

Refer to Section 10.9 for sample size calculations.

5.1.1 Study Diagram

The trial design is depicted in Figure 1.



M= month; V1, V2, etc, = Visit 1, Visit 2, etc; Vacc = vaccination visit
SAEs = serious adverse events; NAbs= neutralizing antibodies, CMI= cell-mediated immunity; cCMVi = congenital CMV infection; ELISPOT = Enzyme-linked immunosorbent spot
Monthly contacts through M36 will be conducted for self-collection reminder (saliva and urine) and to assess pregnancy status. During pregnancy participants will have another monthly contact to assess pregnancy and infant outcome.

[a] Participants in the V160 2-dose regimen arm will receive V160 at Vacc 1, Placebo at Vacc 2, and V160 at Vacc 3.
[b] Any time a pregnancy is reported the participant will be asked to return to the clinic for 2 or 3 visits - one to provide information on pregnancy monitoring and infant follow-up and 1 or 2 others to bring in the infant for collection of infant saliva and urine sample for CMV PCR testing. Infants will be screened for cCMVi by collecting saliva and urine for CMV PCR within 2 weeks of birth. cCMVi positive infants will be monitored for up to 3 years after birth.

Figure 1 Trial Design Diagram

5.2 Number of Participants

Approximately 2100 participants will be randomized in order to accumulate the required number of CMVi cases to provide sufficient power for the primary and secondary efficacy hypotheses.

5.3 Beginning and End of Study Definition

The overall study begins when the first participant signs the informed consent/assent form (ICF). The overall study ends when the last participant completes the last study-related contact or visit, withdraws from the study or is lost to follow-up (ie, the participant is unable to be contacted by the investigator). For purposes of analysis and reporting, the overall study ends when the Sponsor receives the last laboratory result or at the time of final contact with the last participant, whichever comes last.

5.3.1 Clinical Criteria for Early Study Termination

An eDMC will be formed to monitor accumulating safety data from this study. They will also review the results of the IA of the primary endpoint. At the IA for testing the primary efficacy hypothesis, the eDMC may recommend termination of the study for demonstration of futility (as described in Section 10.7). The eDMC may also recommend termination of the study for safety if concerning safety trends are detected. Guidance relating to recommendations for temporary pause or termination of the study will be provided in the eDMC charter that will be written for this study.

5.4 Scientific Rationale for Study Design

This study is designed to evaluate the efficacy of V160 in preventing CMVi in women of childbearing age who are seronegative for CMV prior to vaccination. The primary reason to vaccinate women against CMV is to prevent cCMVi and cCMVd in infants of women who become infected with CMV during pregnancy. A CMV seronegative woman infected during pregnancy has a high probability of transmitting CMV to her fetus (~40%). Congenital CMVi and cCMVd are rare endpoints which are difficult to assess in clinical studies. Therefore, the primary efficacy endpoint in this study is prevention of CMVi in CMV seronegative women. It is postulated that if a woman is durably protected against CMV infection, she should continue to be protected during any future pregnancies thereby eliminating the potential to transmit CMV to her fetus and cases of cCMVi/cCMVd. Although a CMV seropositive woman can also transmit CMV to her fetus, the probability of transmission is much lower (~1-2%), and the sequelae in the infected infant, less severe. Whether this transmission, in CMV seropositive women, occurs as a result of acquisition of a new CMVi or reactivation of a latent infection is unknown.

Since persons who are CMV seropositive (latently infected with the virus) are known to shed the virus intermittently, this study will include only CMV seronegative women in order to optimize demonstration of vaccine efficacy against CMVi. Additional studies in the V160 development program will evaluate the safety and immunogenicity of V160 among CMV seropositive women.

Additionally, women who have exposure to a child/children ≤5 years old at home or occupationally will be included in the study to increase the risk of incident CMVi. As women with young children may be breastfeeding, breastfeeding a child will not be an exclusion criterion for vaccination in the study. Breastfeeding is not a contraindication for other live virus vaccines - Measles, Mumps, Rubella vaccine (M-M-R™ II) or Varicella virus vaccine (Varivax™). The position of the Advisory Committee on Immunization Practices (ACIP) regarding vaccines and breastfeeding is, “Neither inactivated nor live virus vaccines

administered to a lactating woman affect the safety of breast-feeding for mothers of infants." and "Although live vaccines multiply within the mother's body, the majority have not been demonstrated to be excreted in human milk"[Centers for Disease Control and Prevention 2002]. Similarly, for V160, as replication of the virus within the mother is not expected, and shedding has not been detected in saliva or urine, presence of V160 in breast milk is unlikely.

This relatively large Phase 2b study is designed to allow a rapid and robust demonstration of clinical efficacy of V160, with a high probability of success at the IA when the VE ≥85%. The rigor of the analysis criteria is intended to reduce the risk of a false-negative study in the case that the vaccine is truly efficacious.

The 2-dose arm is included to allow for evaluation of a schedule that would optimally coincide with the current adolescent vaccination schedule, allowing maximum uptake when licensed.

5.4.1 Rationale for Endpoints

5.4.1.1 Rationale for Efficacy Endpoint

CMVi (the primary endpoint for this study) is largely asymptomatic in adults, but the virus can be shed in saliva or urine for 6-8 weeks (or perhaps longer) in persons who are infected. Therefore, detection of CMV in the saliva or urine of previously uninfected women will be used to identify cases of incident CMV infection. Sampling of saliva and urine will be conducted monthly, and self-collection will be used in order to encourage compliance. Adequacy of sampling during self-collection will be assessed by evaluation of the samples for a human housekeeping gene, beta-globin, by polymerase chain reaction (PCR) for a period of time after the start of the study (eg, 6 months).

Prior CMV vaccine studies identified incident CMVi by detecting new immunity to CMV and confirming by urine or saliva viral detection. In these studies, serum, urine, and saliva sampling was conducted every 3 months and urine/saliva detection identified nearly all cases of infection [Pass, R. F., et al 2009]. As immunity to V160 is designed to mimic natural immune responses, postdose immune responses cannot be used to identify incident CMVi. Therefore, the focus of this study will be CMV detection in saliva and urine by PCR and the frequency of assessment has been increased from every 3 months to monthly.

If CMV is identified by PCR in a participant, the virus will be typed to determine whether it is vaccine or non-vaccine CMV strain. Since this is the first study to use monthly sampling of saliva and urine to identify incident CMVi, the performance of the sampling methodology will be carefully monitored. If it is determined that reduced sampling frequency or use of a single matrix (saliva or urine) could adequately identify endpoint CMVi cases, modifications to the sampling methodology can be implemented in future studies.

5.4.1.2 Rationale for Immunogenicity Endpoints

The rationale for the immunogenicity endpoint is to characterize the humoral and CMI responses to V160 and explore the relationship between immune responses to V160 and efficacy of the vaccine.

Robust and balanced humoral and cellular CMV-specific immune responses have been found to play an important role in the protection against cCMVi [Plotkin, S. A., et al 1989] [Adler, S. P., et al 1995] [Wang, D., et al 2016].

The purpose of the CMV-specific NAb assay is to assess humoral immunity by quantifying neutralizing antibody titers to CMV before and after vaccination. This assay provided the primary serological readout in Phase 1, allowed quantitative comparison of responses to different doses, and provides a functional characterization of the vaccine response.

For an additional measure of the humoral response, an IgG ELISA assay being will be used to measure the total IgG response to V160 as a secondary serological read-out. This assay will also be evaluated as a surrogate of the functional neutralization assay.

The purpose of the CMV ELISPOT assay is to assess CMI responses before and after vaccination. This assay was also used in Phase 1 and while robust responses were demonstrated, it did not allow quantitative comparison between groups. It will be performed on only a subset of participants to confirm CMI responses to V160.

5.4.1.3 Rationale for Safety Endpoints

The safety endpoints evaluated in this study were selected based on the product's safety profile demonstrated in the Phase 1 study, and on feedback received from regulatory agencies during product development.

In this study, as in the Phase I study, pre-specified solicited systemic AEs include tiredness (fatigue), muscle pain (myalgia), headache, and joint pain (arthralgia) and will be collected Days 1 to 14 following each dose of V160/placebo. The vaccine has limited potential for replication, therefore, an extended collection time of safety follow-up typically used for live virus vaccines is not warranted in this study.

The timepoints chosen for safety assessment are based on peak occurrence of AEs generally observed following vaccination in clinical trials with multi-dose vaccines.

AEs will be assessed and graded for intensity/toxicity per Guidance for Industry. Although death is not included in the Toxicity Grade Guidance for Industry, for this protocol, any death will be assessed as Toxicity Grade 4.

Details on AEs, including definitions and reporting requirements, can be found in Appendix 4.

5.4.1.4 Rationale for Congenital CMVi Exploratory Endpoint

V160 is being developed to prevent CMVi in women of childbearing potential and thereby cCMVi in their infants. Evaluation of infant outcomes of any women who become pregnant is an exploratory objective in this trial. As there is no routine screening for CMVi in pregnancy or cCMVi at birth, the study will collect a woman's prenatal care records for evidence of suspicion of congenital infection (eg, ultrasounds with increased placental thickness, fetal growth retardation, amniocentesis procedures looking for CMV, etc.). When a pregnancy is not carried to term, data on spontaneous abortions and terminations will be collected. Additionally, suspicion for congenital infections at birth will be determined from review of delivery records. Infants will be screened for cCMVi in the study by collecting

saliva and urine for CMV PCR within 3 weeks of birth. When there is a suspicion or diagnosis of cCMVi outside the study, or infant samples are collected outside the study window, endpoint determination for cCMVi will be performed by an independent, blinded, clinical adjudication committee.

Refer to Section 9.2.1.2 for cCMVi endpoint assessment including case definition and detection. Details of the cCMVi exploratory endpoint analysis will be provided in the supplementary statistical analysis plan (sSAP).

5.4.1.5 Planned Exploratory Biomarker Research

5.4.1.5.1 Future Biomedical Research

The Sponsor will conduct Future Biomedical Research on DNA specimens for which consent was provided during this clinical study. This research may include genetic analyses (DNA), gene expression profiling (ribonucleic acid [RNA]), proteomics, metabolomics (serum, plasma) and/or the measurement of other analytes, depending on which specimens are consented for future biomedical research.

Such research is for biomarker testing to address emergent questions not described elsewhere in the protocol (as part of the main study) and will only be conducted on specimens from appropriately consented participants. The objective of collecting/retaining specimens for Future Biomedical Research is to explore and identify biomarkers that inform the scientific understanding of diseases and/or their therapeutic treatments. The overarching goal is to use such information to develop safer, more effective drugs/vaccines, and/or to ensure that participants receive the correct dose of the correct drug/vaccine at the correct time. The details of this Future Biomedical Research sub-study are presented in Appendix 2.

5.4.1.6 Rationale for the Use of Comparator/Placebo

A concurrent placebo arm (sterile saline) is included in this study in order to provide CMV infection rates for determination of vaccine efficacy.

There is currently no active comparator licensed for the prevention of CMV infection

5.4.1.7 Rationale for Adjuvant

The Merck Aluminum Phosphate Adjuvant (MAPA) was assessed in the Phase 1 trial in which formulations containing MAPA tended to be more immunogenic than those without, particularly at the 100-unit dose. Therefore, MAPA is included in the V160 formulation for this Phase 2 trial. The dose level of MAPA in the V160 formulation to be used in this trial is consistent with the amount of aluminum phosphate adjuvant (125 µg/dose to 500 µg/dose) used in many other licensed vaccines.

5.5 Justification for Dose

The V160 formulation selected for this trial is 100 Units/0.5 mL dose +225 µg of MAPA as this formulation provided the greatest immunogenicity (measured by the NAb response) in the Phase 1 trial. All participants randomized to either of the two vaccine groups will be given either 2 or 3 injections (depending on randomization) of the V160 100 units/0.5 mL +MAPA. The maximum dose at each dosing time point is the same as the starting dose.

5.5.1 Rationale for Dose Interval and Trial Design

The proposed dosing interval is intended to provide early induction of CMV-specific immune responses (with 2 "priming" doses given within 2 months) and establishment of long-term immunogenicity by providing a third "boost" dose at a later time (6 months). A 3-dose schedule of V160 administered at Day 1, Month 1, and Month 6 was used for the Phase 1 study. For this Phase 2b study, a Day 1, Month 2, and Month 6 schedule and a Day 1, Month 6 schedule were selected. Since the 0, 1, 6 month and 0, 2, 6-month regimens are designed with 2 priming doses followed by a boost dose, it is not anticipated that the change from 1 to 2 months for the second dose in the 3-dose regimen will impact immunogenicity. The 2-dose regimen with a single priming dose followed by a boost dose is being evaluated because it would allow greater compliance. Both these regimens would allow alignment with other adolescent vaccines such as Gardasil™ and Gardasil™ 9.

6. Study Population

Participants will be healthy, CMV seronegative, non-pregnant, female participants of child-bearing potential, age 16-35 years.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Healthy, based on medical history and physical examination
2. Female, 16 to 35 years of age
3. Serologically confirmed as CMV seronegative (based on CMV IgG) prior to receiving the first dose of V160/placebo
4. Have direct exposure to young children (≤5 years of age) at home or occupationally
5. Childbearing potential (see definition of women of childbearing potential [WOCBP] in Appendix 3)
6. Agrees to avoid becoming pregnant during the 6-month treatment phase and for 4 weeks after the last dose of V160/placebo (from Day 1 through Month 7) by either practicing abstinence from heterosexual activity OR by using allowable methods of birth control during heterosexual activity. Allowable methods of birth control are provided in the Appendix 3.

Informed Consent/Assent

7. Voluntarily agrees to participate (or legally acceptable representative, for minors, if applicable); and provide written informed consent/assent for the trial. The participant may also provide consent/assent for Future Biomedical Research. However, the participant may participate in the main trial without participating in Future Biomedical Research.

Protocol Adherence

8. Be able to complete all scheduled visits and to comply with the study procedures
9. Agrees to provide study personnel with a primary telephone number as well as an alternate form of contact, if available, for follow-up purposes

6.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Has a history or current evidence of any condition, therapy, laboratory abnormality or other circumstance that might expose the participant to risk by participating in the trial, confound the results of the trial, or interfere with participation for the full duration of the trial, as assessed by the investigator
2. Has a history of any allergic reaction or an anaphylactic/anaphylactoid reaction to any vaccination that required medical intervention, or of any severe allergic reaction (eg, swelling of the mouth and throat, difficulty breathing, hypotension, or shock), to any vaccine component that required medical intervention
3. Has a recent (<72 hours) history of febrile illness (temperature ≥100.4°F/38.0°C, oral equivalent)
4. Is currently immunocompromised or has been diagnosed as having a congenital or acquired immunodeficiency, human immunodeficiency virus (HIV) infection, lymphoma, or leukemia. Participants with autoimmune conditions that require immunosuppressive medications (eg, systemic lupus erythematosus, rheumatoid arthritis, juvenile rheumatoid arthritis, inflammatory bowel disease) are also to be excluded. No testing for HIV will be required in the study.
5. Has a condition in which repeated venipuncture or injections pose more than minimal risk for the participant, such as hemophilia, thrombocytopenia, other severe coagulation disorders, or significantly impaired venous access
6. A woman of childbearing potential (WOCBP) (see Appendix 3) who has a positive pregnancy test at screening or within 24 hours before the first dose of study treatment. If the urine test on the day of vaccination cannot be confirmed as negative, a serum pregnancy test is required. In such cases, the participant must be excluded from participation if the serum pregnancy result is positive. If pregnancy is diagnosed any time after the first dose but before the completion of the vaccination series, the participant is excluded from any remaining vaccinations but is included in the study for safety follow-up.

Prior/Concomitant Therapy

7. Has previously received a CMV vaccine
8. Had any live virus vaccine administered or scheduled to be administered in the period from 4 weeks prior to, and 4 weeks following receipt of any dose of trial vaccine
9. Had any inactivated vaccine administered or scheduled within the period from 14 days prior to, through 14 days following, any dose of trial vaccine
10. Had administration of any immune globulin or blood product within 90 days prior to injection with V160/placebo or scheduled within 30 days thereafter
11. Has received systemic corticosteroids (equivalent of ≥2 mg/kg total daily dose of prednisone or ≥20 mg/d for persons weighing >10 kg) for ≥14 consecutive days and has not completed treatment at least 30 days prior to trial entry
12. Has received systemic corticosteroids exceeding physiologic replacement doses (≈5 mg/d prednisone equivalent) within 14 days prior to the first vaccination (participants using inhaled, nasal, or topical steroids are considered eligible for the trial)
13. Has received any anti-viral agent with proven or potential activity against CMV two weeks prior to vaccination or is likely to receive such an agent within 2 weeks after vaccination. Anti-viral agents prohibited include letermovir, ganciclovir, valganciclovir, foscarnet, cidofovir, and valacyclovir.
14. Is receiving or has received in the year prior to enrollment immunosuppressive therapies including but not limited to rapamycin (also sirolimus), tacrolimus (also FK-506 or Fujimycin), or other therapies used for solid organ/cell transplant, radiation therapy, immunosuppressive/cytotoxic immunotherapy, chemotherapy and other immunosuppressive therapies known to interfere with the immune response. Topical tacrolimus is allowed provided that it is not used within 2 weeks prior to, or 2 weeks following a V160 dose.

Prior/Concurrent Clinical Study Experience

15. Has participated in another clinical trial in the past 4 weeks or plans to participate in a treatment-based trial or a trial in which an invasive procedure is to be performed while enrolled in this trial. (Participation in a safety surveillance or non-interventional trial is acceptable).

Other Exclusions

16. Planned donation of eggs at any time from signing the informed consent through 1 month after receiving the last dose of the trial V160/placebo
17. Is or has an immediate family member (eg, spouse, parent/legal guardian, sibling or child) who is investigational site or sponsor staff directly involved with this study
18. Any other reason that in the opinion of the investigator might interfere with the evaluation required by the study

6.2.1 Participant Deferment Criteria before Vaccination

These deferment criteria must be reviewed for each participant before each vaccination to ensure that none of the criteria apply to the participant and or that vaccination is deferred for an appropriate period of time. The participant should be deferred if:

1. Has a recent (<72 hours) history of febrile illness (temperature ≥100.4ºF/38.0°C, oral equivalent)
2. Had any live virus vaccine administered or scheduled to be administered in the period from 4 weeks prior to, through 1 month following receipt of any dose of study vaccine
3. Had any inactivated vaccine administered or scheduled within the period from 14 days prior to, through 14 days following, any dose of trial vaccine
4. Has received systemic corticosteroids (equivalent of ≥2 mg/kg total daily dose of prednisone or ≥20 mg/d for persons weighing >10 kg) for ≥14 consecutive days and has not completed treatment at least 30 days prior to any dose of trial vaccine
5. Has received systemic corticosteroids exceeding physiologic replacement doses (≈5 mg/d prednisone equivalent) within 14 days prior to the any dose of trial vaccine (use of inhaled, nasal, or topical steroids is allowed for the trial)
6. Had administration of any immune globulin or blood product within 90 days prior to injection with V160/placebo or scheduled within 30 days thereafter
7. Has received any anti-viral agent with proven or potential activity against CMV 2 weeks prior to vaccination or is likely to receive such an agent within 2 weeks after vaccination. Anti-viral agents prohibited include letermovir, ganciclovir, valganciclovir, foscarnet, cidofovir, and valacyclovir
8. Has received or is scheduled to receive topical tacrolimus within 2 weeks prior to or 2 weeks following a V160 dose.

6.2.2 Criteria to be Checked for Excluding Participants from Receiving Subsequent Doses

Specific exclusion criteria must be reviewed for each participant before administration of any subsequent dose of V160/placebo to ensure that none of the criteria apply to the participant. If any apply, the participant will be excluded from receiving the scheduled vaccination (and therefore be excluded from the per protocol analysis) but will continue in the study for all other follow-up procedures. A complete list of criteria to be checked prior to Dose 2 and Dose 3 are provided in Section 8.1.

6.3 Lifestyle Restrictions

No lifestyle restrictions are required.

6.3.1 Meals and Dietary Restrictions

No specific meal and/or dietary restrictions are required.

6.3.2 Caffeine Alcohol, and Tobacco Restrictions

No specific caffeine, alcohol, or tobacco restrictions are required.

6.3.3 Activity

No specific activity restrictions are required.

6.4 Screen Failures

Screen failures are defined as participants who consent/assent to participate in the clinical study but are not subsequently randomized in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any adverse events or serious adverse events (SAE) meeting reporting requirements as outlined in the data entry guidelines.

6.5 Participant Replacement Strategy

A participant who discontinues from trial treatment/vaccination or withdraws from the trial will not be replaced.

7. Treatments

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

Clinical supplies [study treatment(s) provided by the Sponsor] will be packaged to support enrollment. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

7.1 Treatments Administered

The study treatments to be used in this trial are outlined below in Table 1.

Table 1 Study Treatments

Study Treatment Name:	V160	MAPA Diluent	Sterile Saline Placebo
Dosage Formulation:	Lyophilized Cake	Sterile Suspension for reconstitution	Sterile Solution
Unit Dose Strength:	200 Units/mL	450 µg/mL	*Not applicable*
Dosage Level:	100 Units with 225 µg aluminum per 0.5mL dose		0.5mL
Route of Administration:	IM injection		IM injection
Sourcing:	Sponsor	Sponsor	Sponsor

MAPA Diluent= V160 Merck Aluminum Phosphate Adjuvant (MAPA) Diluent
Sterile Saline Placebo = V160 Sterile Saline Placebo
Unit dose strength= strength of the V160 and MAPA products after reconstitution
Dosage level = post reconstitution per 0.5mL dose

All supplies indicated in Table 1 will be provided per the 'Sourcing' row depending upon local country operational requirements. Every attempt should be made to source these supplies from a single lot/batch number. The trial site is responsible for recording the lot number, manufacturer, and expiry date for any locally purchased product as per local guidelines unless otherwise instructed by the Sponsor.

Refer to section 9.1.8 for details regarding administration of the study treatment.

7.2 Dose Modification

All vaccine doses will be 0.5 mL (100 Units+MAPA): no modifications will be made during the trial.

7.3 Method of Treatment Assignment

All eligible participants will be randomly allocated and will receive a treatment/randomization number. The treatment/randomization number identifies the participant for all procedures occurring after treatment allocation/randomization. Once a treatment/randomization number is assigned to a participant, it can never be re-assigned to another participant. A single participant cannot be assigned more than 1 treatment/randomization number.

Treatment allocation/randomization will occur centrally using interactive response technology (IRT). There are 3 study treatment arms. Participants will be assigned randomly in a 1:1:1 ratio.

Furthermore, a subset of study participants will be enrolled to participate in a Detailed Immunogenicity sub-study. Sub-study participants will be asked to provide an additional blood sample for NAbs and IgG ELISA antibodies at Month 6, prior to the last vaccination,

and blood samples for PBMC isolation to allow for assessment of CMI response (CMV IFN-γ ELISPOTs) at timepoints shown in the SoA.

Detail Immunogenicity sub-study participants will be recruited from sub-study sites, which are sites that are near a validated facility that can process blood samples for PBMCs. Non-sub-study sites are clinical study sites that are not near a validated facility that can process blood samples for PBMCs. Study participants enrolled in non-sub-study clinical sites are ineligible to participate in the Detailed Immunogenicity sub-study.

The randomization ratios relating to allocation of study participants into the 3 treatment arms and the sub-study are shown in Table 2.

Table 2 Allocation Ratio across Treatment Arms and Detailed Immunogenicity Sub-Study

	Allocation Ratio (N)		
	V160 3-Dose (Group A)	V160 2-Dose (Group B)	Placebo (Group C)
All Participants	1 (700)	1 (700)	1 (700)
Sub-study participants	1 (120)	1 (120)	1 (120)
Sub-study non-participants	1 (580)	1 (580)	1 (580)

N = Sample size

Approximately 360 study participants (or as many as are operationally feasible) will be enrolled in the sub-study, to be allocated in a 1:1:1 ratio in each of the 3-dose regimen, 2-dose regimen, and placebo arms. For operational simplicity, the participants of the sub-study will be selected as follows:

- Starting at the time when the facility that can process blood samples for PBMCs near a particular sub-study site is validated, study sites near the facility become a sub-study site subject to the capacity of the facility. Study participants randomized into the study by this particular sub-study site will become participants in the Detailed Immunogenicity sub-study. A site may choose to withdraw from participation in the sub-study after approval from the Sponsor. Participants of the Detailed Immunogenicity sub-study will be recruited in this manner until approximately 360 sub-study participants (or as many as are operationally feasible) across all sub-study sites have been enrolled.
    - Sites may randomize participants into the study prior to the time the PBMC blood sample processing facility is validated; however, these participants will not be included in the Detailed Immunogenicity sub-study.
- Additional study participants enrolled by sub-study sites over and above 360 sub-study participants will not be enrolled in the sub-study.

In order to increase the likelihood that approximately 360 participants enrolled into the study by sub-study sites are allocated approximately equally into the 3 treatment arms, two allocation schedules will be generated; one for use by non-sub-study sites and another for use by sub-study sites.

Randomization will stop when ≈2100 participants have been randomized into the study.

7.3.1 Stratification

No stratification based on age, sex, or other characteristics will be used in this trial.

7.4 Blinding

A double-blinding technique with in-house blinding will be used. V160 and placebo will be prepared and administered in a blinded fashion by an unblinded pharmacist or qualified trial site personnel because the two products are visually different (the vaccine is opaque, and the placebo is clear). The participant and the investigator who is involved in the clinical evaluation of the participants are unaware of the group assignments.
In order to avoid bias, the unblinded individual/s will not be involved in any postdose safety assessment procedures. The unblinded individual/s also must not disclose any information regarding the allocation of the clinical supplies or the appearance of the V160/placebo to any blinded member of the site staff or to the participant. No blinded member of the site staff should have contact with the clinical supplies at any point during the course of the study. Note that participants must be discontinued from vaccination if they become unblinded (Section 8.1).

See Section 9.1.10 for a description of the method of unblinding a participant during the trial, should such action be warranted.

7.5 Preparation/Handling/Storage/Accountability

7.5.1 Dose Preparation

As described in Section 7.4, unblinded personnel will be responsible for reconstitution of study vaccine in order to produce the final study vaccine formulation that will be administered to the participant. Blinded personnel will not be present during vaccine administration.

Reconstitution of V160 Lyophilized Cake:

Reconstitution of the V160 lyophilized cake should be completed within 90 minutes after removal from the refrigerated storage unit.

1. Remove V160 and V160 MAPA diluent vials from the refrigerated storage unit.
2. Mix the contents of the V160 MAPA diluent vial by shaking thoroughly to get a homogeneous mixture.
3. Using a syringe and needle, withdraw 0.7 mL of V160 MAPA diluent from the diluent vial, and inject immediately into the V160 lyophilized cake vial.

4. Gently swirl the V160 vial and examine for undissolved material. Continue swirling until the lyophilized cake has completely dissolved and the appearance is opalescent. Disregard any bubbles that may be present.
5. Withdraw and administer 0.5 mL of the reconstituted V160 vial.

Study vaccine should be administered immediately following dose preparation. If the reconstituted product is not used immediately, the vial/syringe contents should be mixed by inverting several times to get a homogeneous mixture of the product immediately prior to use. If the study vaccine or placebo is not administered within 60 minutes, it should be disposed of as a biohazardous medical waste and a new study vaccine should be prepared.

Preparation of Placebo

The sterile saline placebo will be used as supplied.

7.5.2 Handling, Storage and Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

For all trial sites, the local country Sponsor personnel or designee will provide appropriate documentation that must be completed for drug accountability and return, or local discard and destruction if appropriate. Where local discard and destruction is appropriate, the investigator is responsible for ensuring that a local discard/destruction procedure is documented.

The trial site is responsible for recording the lot number, manufacturer, and expiry date for any locally purchased product as per local guidelines unless otherwise instructed by the Sponsor.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of study treatments in accordance with the protocol and any applicable laws and regulations.

7.6 Treatment Compliance

Trial compliance is defined in this study as participants who received all 3 doses of scheduled study vaccinations/placebo.

Interruptions from the protocol specified vaccination plan require consultation between the investigator and the Sponsor and written documentation of the collaborative decision on participant management.

7.7 Concomitant Therapy

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during time periods specified by this protocol for the particular medication or vaccination.

If there is a clinical indication for any medication or vaccination specifically prohibited, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Sponsor Clinical Director. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician. However, the decision to continue the participant on study treatment requires the mutual agreement of the investigator, the Sponsor and the participant.

See the exclusion criteria for specific restrictions for prior and concomitant medications at Day 1 (Section 6.2) and prerequisites for other vaccination visits (Section 6.2.2).

Participants may receive allergen desensitization therapy and tuberculin skin testing while participating in the study.

Topical tacrolimus is allowed provided that it is not used 2 weeks prior to, or 2 weeks following a V160 dose.

Use of prior and concomitant medications/vaccination should be recorded as described in Section 9.1.5.

7.7.1 Rescue Medications and Supportive Care

No rescue or supportive medications are specified to be used in this trial.

7.8 Treatment After the End of the Study

There is no study-specified treatment following the end of the study.

7.9 Clinical Supplies Disclosure

This trial is blinded but supplies are provided open label; therefore, an unblinded pharmacist or qualified trial site personnel will be used to blind supplies. Study treatment identity (name, strength or potency) is included in the label text; random code/disclosure envelopes or lists are not provided.

The emergency unblinding call center will use the treatment/randomization schedule for the trial to unblind participants and to unmask study treatment identity. The emergency unblinding call center should only be used in cases of emergency (see Section 9.1.10). In the event that the emergency unblinding call center is not available for a given site in this trial, the interactive response technology (IRT) should be used in order to unblind participants and to unmask study treatment identity. The Sponsor will not provide random code/disclosure envelopes or lists with the clinical supplies.

See Section 9.1.10, Participant Blinding/Unblinding, for a description of the method of unblinding a participant during the trial, should such action be warranted.

8. Discontinuation/Withdrawal Criteria

8.1 Discontinuation of Study Treatment

Discontinuation of study treatment does not represent withdrawal from the study.

As certain data on clinical events beyond study treatment discontinuation may be important to the study, they must be collected through the participant's last scheduled follow-up, even if the participant has discontinued study treatment. Therefore, all participants who discontinue study treatment prior to completion of the vaccination regimen will still continue to participate in the study as specified in the SoA. This includes the self-collection of urine and saliva samples by study participants.

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Sponsor if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at study treatment discontinuation are provided in Section 9.1.9.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment.
- The participant's treatment assignment has been unblinded by the investigator, Merck subsidiary or through the emergency unblinding call center.
- The participant has a medical condition or personal circumstance which, in the opinion of the investigator and/or sponsor, places the participant at unnecessary risk from continued administration of study treatment.
- The participant has a confirmed positive pregnancy test.
- The participant had an allergic reaction or an anaphylactic/anaphylactoid reaction that required medical intervention following Dose 1 administration.
- The participant enrolled in another interventional clinical trial following Dose 1 administration.
- Received a non-study CMV vaccine.

For participants who are discontinued from study treatment but continue to be monitored in the trial, all visits and procedures, as outlined in the SoA, should be completed.

Discontinuation from study treatment is "permanent." Once a participant is discontinued, she shall not be allowed to restart study treatment.

8.2 Withdrawal from the Study

A participant must be withdrawn from the study if the participant or participant's legally acceptable representative withdraws consent from the study.

If a participant withdraws from the study, they will no longer receive study treatment or be followed at scheduled protocol visits.

Specific details regarding procedures to be performed at the time of withdrawal from the study, as well as specific details regarding withdrawal from Future Biomedical Research are outlined in Section 9.1.9. The procedures to be performed should a participant repeatedly fail to return for scheduled visits and/or if the study site is unable to contact the participant are outlined in Section 8.3.

8.3 Lost to Follow Up

If a participant fails to return to the clinic for a required study visit and/or if the site is unable to contact the participant, the following procedures are to be performed:

- The site must attempt to contact the participant and reschedule the missed visit. If the participant is contacted, the participant should be counseled on the importance of maintaining the protocol-specified visit schedule.
- The investigator or designee must make every effort to regain contact with the participant at each missed visit (eg, phone calls and/or a certified letter to the participant's last known mailing address or locally equivalent methods). These contact attempts should be documented in the participant's medical record.
- Note: A participant is not considered lost to follow-up until the last scheduled visit for the individual participant. The amount of missing data for the participant will be managed via the pre-specified data handling and analysis guidelines.

9. Study Assessments and Procedures

- Study procedures and their timing are summarized in the SoA.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- The Investigator is responsible for assuring procedures are conducted by staff appropriately qualified by education, training or experience. Delegation of study site personnel responsibilities will be documented in the Investigator Trial File Binder (or equivalent).
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and were performed within the time frame defined in the SoA.

- Additional evaluations/testing may be deemed necessary by the investigator and or the Sponsor for reasons related to participant safety. In some cases, such evaluation/testing may be potentially sensitive in nature (eg, HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent, and assent if applicable, be obtained from the participant. In these cases, such evaluations/testing will be performed in accordance with those regulations.

The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required is shown in Table 3.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Table 3 Approximate Blood/Tissue Volumes Drawn/Collected by Trial Visit and by Sample Types

Population	**Sample**	**Visit Number, Time point**	**Approximate Volume Collected**
All participants	Serum for CMV serostatus by IgG	Visit 1, Screening	3.5 mL
	Serum β-Human Chorionic Gonadotropin (β-hCG)	Visit 1, Screening	3.5 mL
	Blood (DNA) for Future Biomedical Research	Visit 2, Day 1	8.5 mL
	Serum for Neutralizing Antibody Serum for IgG ELISA antibodies	Visit 2, Day1	15 mL
		Visit 5, Month 7	15 mL
		Visit 6, Month 12	15 mL
		Visit 8, Month 24	15 mL
		Visit 10, Month 36	15 mL
	Non-sub Study Participants Expected Total Volume Collected		90.5 mL
Detailed Immunogenicity sub-study participants only[a]	Serum for Neutralizing Antibody Serum for IgG ELISA antibodies	Visit 4, Month 6	15 mL
	Blood for ELISPOT (PBMCs)	Visit 2, Day1	50 mL
		Visit 4, Month 6	50 mL
		Visit 5, Month 7	50 mL
		Visit 6, Month 12	50 mL
		Visit 8, Month 24	50 mL
		Visit 10, Month 36	50 mL
	Additional Sub-Study Expected Total Volume Collected		315 mL
	Sub-study Participants Expected Total Volume Collected (all visits included)		405.5 mL

[a] Note that these samples are **in addition to** samples collected for all participants.

9.1 Administrative and General Procedures

9.1.1 Informed Consent/Assent

The investigator or qualified designee must obtain documented consent, and assent if applicable, from each potential participant or each participant's legally acceptable representative prior to participating in a clinical trial or Future Biomedical Research. If there are changes to the participant's status during the trial (eg, health or age of majority requirements), the investigator or qualified designee must ensure the appropriate consent/assent is in place.

9.1.1.1 General Informed Consent/Assent

Consent/assent must be documented by the participant's dated signature or by the participant's legally acceptable representative's dated signature on a consent/assent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent/assent form should be given to the participant before participation in the trial.

The initial informed consent/assent form, any subsequent revised written informed consent/assent form and any written information provided to the participant must receive the IRB/IEC's approval/favorable opinion in advance of use. The participant or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the participant's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent/assent form or addendum to the original consent/assent form that captures the participant's dated signature or by the participant's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent/assent form template at the protocol level.

The informed consent will adhere to IRB/IEC requirements, applicable laws and regulations and Sponsor requirements. The assent, as applicable will adhere to IRB/IEC requirements, applicable laws and regulations and Sponsor requirements.

Pregnancy follow-up will be included in the general informed consent form in the event that any participant becomes pregnant, and will allow the site to monitor the pregnancy, and have access to medical records pertaining to the pregnancy and the infant outcome.

A separate informed consent will be requested for any participant who becomes pregnant during the study. This consent will be obtained when the pregnancy is reported and a visit is scheduled for pregnancy and infant follow-up (at cCMVi Visit 1).

9.1.1.2 Consent/Assent and Collection of Specimens for Future Biomedical Research

The investigator or qualified designee will explain the Future Biomedical Research consent/assent to the participant, answer all of his/her questions, and obtain written informed consent/assent before performing any procedure related to the Future Biomedical Research sub-trial. A copy of the informed consent/assent will be given to the participant.

9.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the participant qualifies for the study. An additional review of exclusion/discontinuation criteria will be done at subsequent doses after Dose 1. These criteria are listed in Section 8.1.

9.1.3 Participant Identification Card

All participants will be given a Participant Identification Card identifying them as participants in a research study. The card will contain trial site contact information (including direct telephone numbers) to be utilized in the event of an emergency. The investigator or qualified designee will provide the participant with a Participant Identification Card immediately after the participant provides written informed consent/assent. At the time of treatment allocation/randomization, site personnel will add the treatment/randomization number to the Participant Identification Card.

The participant identification card also contains contact information for the emergency unblinding call center so that a health care provider can obtain information about study treatment in emergency situations where the investigator is not available.

9.1.4 Medical History

A medical history will be obtained by the investigator or qualified designee at the screening visit in order to ensure that the participant satisfies the inclusion and exclusion criteria of the study. The participant's medical conditions will be reported on the appropriate eCRF, including any chronic or serious conditions.

9.1.5 Prior and Concomitant Medications Review

9.1.5.1 Prior Medications

The investigator or qualified designee will review prior medication/vaccination use and record prior medication taken by the participant to assess inclusion and exclusion criteria including time windows for medication/vaccination use.

9.1.5.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the participant during the trial. As outlined in the exclusion criteria, there are specific restrictions for prior medications and vaccines, as well as concomitant medications and vaccines that are anticipated throughout the duration of the study. Investigators should make every attempt to adhere to the entry criteria concerning prior, concomitant, and anticipated medications and vaccines.

9.1.6 Assignment of Screening Number

All consented participants will be given a unique screening number that will be used to identify the participant for all procedures that occur prior to randomization. Each participant will be assigned only one screening number. Screening numbers must not be re-used for different participants.

Any participant who is screened multiple times will retain the original screening number assigned at the initial screening visit.

Note that participants who meet screening eligibility may be randomized outside of the 21-day window; however, all screening procedures must be repeated when a participant is not randomized within 21 days of Visit 1 (screening). Participants who are re-screened must be randomized within 21 days of the re-screening visit.

9.1.7 Assignment of Treatment/Randomization Number

All eligible participants will be randomly allocated and will receive a treatment/randomization number. The treatment/randomization number identifies the participant for all procedures occurring after treatment allocation/randomization. Once a treatment/randomization number is assigned to a participant, it can never be re-assigned to another participant.

A single participant cannot be assigned more than 1 treatment/randomization number.

9.1.8 Treatment Administration

V160/placebo should be prepared and administered by appropriately qualified members of the study personnel (eg, physician, nurse, physician's assistant, nurse practitioner, pharmacist, or medical assistant) as allowed by local/state, country, and institutional guidance. The study personnel responsible for vaccine preparation/administration will be unblinded and will have no further contact with the participants or data. Open-label, single-dose vials will be supplied to the unblinded individual(s) at the clinical site. Supplies will be affixed with a clinical label in accordance with regulatory requirements.

Note that the V160/placebo should be administered within 60 minutes of preparation. Refer to Section 7.5.1 for details.

The V160/placebo will be administered intramuscularly in the deltoid muscle using the syringe that was prepared by the unblinded individual/s. Injections should be administered at a 90° angle into the muscle tissue using a needle long enough to ensure intramuscular deposition of V160/placebo. A separate, sterile disposable unit should be used for the administration of V160/placebo to each participant. Needles should not be recapped. Safe disposal procedures should be followed. Study participants will be observed for 30 minutes following each vaccination for any immediate adverse events and longer if necessary. Adequate treatment provision, including epinephrine, should be available for immediate use should an anaphylactic or anaphylactoid reaction occur.

Participants who meet any of the deferred exclusion criteria (Section 6.2.1) may subsequently return to the clinic to determine eligibility status prior to vaccination.

Prior to each subsequent vaccination, exclusion criteria for subsequent vaccination (Section 6.2.2 and Section 8.1) will be assessed.

Any participant who has a positive pregnancy test prior to vaccination, will not be vaccinated.

9.1.8.1 Timing of Dose Administration

Dose 1 of study treatment for all treatment groups will begin on the day of treatment randomization at Visit 2 (Day 1).

For participants randomized to the 3-dose regimen (Day 1, Month 2, and Month 6), a second dose of study treatment will be given at Visit 3 (Month 2) and a third dose will be given at Visit 4 (Month 6).

For participants randomized to the 2-dose regimen (Day 1 and Month 6), placebo will be given at Visit 3 (Month 2), and the second dose will be given at Visit 4 (Month 6).

For participants randomized to the placebo arm, placebo will be given at Visit 2 (Day 1), Visit 3 (Month 2), and Visit 4 (Month 6).

9.1.9 Withdrawal/Discontinuation

When a participant withdraws from participation in the trial, all applicable activities scheduled for the Month 7 visit (if not completed) or the final trial visit should be performed at the time of withdrawal. Any adverse events which are present at the time of withdrawal should be followed in accordance with the safety requirements outlined in Section 9.3 - Adverse Events.

9.1.9.1 Withdrawal From Future Biomedical Research

A Participant's consent for Future Biomedical Research may be withdrawn by the participant or the participant's legally acceptable representative (as appropriate) and their specimens and all derivatives destroyed. A participant's consent may be withdrawn at any time by contacting the principal investigator for the main trial. If medical records for the main trial are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com). Subsequently, the participant's consent for Future Biomedical Research will be withdrawn. A letter will be sent from the Sponsor to the investigator confirming the withdrawal. It is the responsibility of the investigator to inform the participant of completion of withdrawal. Any analyses in progress at the time of request for withdrawal or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (eg, if the investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the participant's personal information and their specimens. In this situation, the request for specimen withdrawal cannot be processed.

9.1.10 Participant Blinding/Unblinding

STUDY TREATMENT IDENTIFICATION INFORMATION IS TO BE UNMASKED ONLY IF NECESSARY FOR THE WELFARE OF THE PARTICIPANT. EVERY EFFORT SHOULD BE MADE NOT TO UNBLIND THE PARTICIPANT UNLESS NECESSARY.

For emergency situations where the investigator or delegate needs to identify the treatment used by a participant and the dosage administered, he/she will contact the emergency unblinding call center by telephone and make a request for emergency unblinding. As requested by the investigator or delegate the emergency unblinding call center will provide the information to him/her promptly and report unblinding to the sponsor. Prior to contacting the emergency unblinding call center to request unblinding of a participant's treatment assignment, the investigator or delegate must enter the toxicity/intensity grade of the adverse events observed, the relation to study treatment, the reason thereof, etc., in the medical chart.

Participants whose treatment assignment has been unblinded by the investigator/delegate and/or non-study treating physician must be discontinued from study treatment, but should continue to be monitored in the trial.

Additionally, the investigator must go into the IRT system and perform the unblind in the IRT system to update treatment disposition. In the event that the emergency unblinding call center is not available for a given site in this trial, IRT should be used for emergency unblinding in the event that this is required for participant safety.

Study treatment identification information is to be unmasked ONLY if necessary for the welfare of the participant. Every effort should be made not to unblind the participant unless necessary.

In the event that unblinding has occurred, the circumstances around the unblinding (eg, date, reason and person performing the unblinding) must be documented promptly, and the Sponsor Clinical Director notified as soon as possible. Once an emergency unblinding has taken place, the principal investigator, site personnel, and Sponsor personnel may be unblinded so that the appropriate follow-up medical care can be provided to the participant.

At the end of the trial, unblinding logs are to be returned to the Sponsor or designee.

9.1.11 Distribution of Electronic Vaccination Report Card

The electronic vaccination report card (eVRC) was developed to be administered electronically via a hand-held device. This item was structured as recommended in the final FDA Patient Reported Outcome (PRO) Guidance. The investigator or delegate will train the participant in the use of the eVRC prior to dispensing it to the participant at Visit 2 (Day 1). Body temperatures, injection site reactions, vaccine specific complaints, other complaints or illnesses and medications/vaccinations will be recorded on the eVRC following each vaccination (refer to Section 9.5.4 for safety assessments using the eVRC).

9.1.12 Calibration of Equipment

The investigator or qualified designee has the responsibility to ensure that any device or instrument used for a clinical evaluation/test during a clinical study that provides information about inclusion/exclusion criteria and/or safety or efficacy parameters shall be suitably calibrated and/or maintained to ensure that the data obtained is reliable and/or reproducible. Documentation of equipment calibration must be retained as source documentation at the study site.

9.2 Efficacy and Immunogenicity Assessments

9.2.1 Efficacy Assessment

Efficacy of the V160 vaccine will be assessed by monitoring for CMVi among study participants (primary endpoint) and cCMVi in pregnancies and/or infants of study participants (exploratory endpoint). This will be done by evaluating self-collected urine and saliva samples from study participants by PCR for CMV and pregnancies and/or infants of study participants for evidence of cCMVi.

9.2.1.1 CMVi

9.2.1.1.1 CMVi Case Definition

A case of CMVi is defined as the detection of CMV (non-vaccine type) by PCR from a single saliva or urine sample in a previously CMV uninfected participant (CMV seronegative and no prior non-vaccine CMV detected) in the per protocol population.

9.2.1.1.2 CMVi Case Detection

At Visit 2 (Day 1), participants will be trained on proper saliva and urine sample collection procedures using the self-collection kits provided. The first set of samples will be collected on site by the participant. All other samples will be collected monthly, from Month 1 to approximately Month 36, by the participant outside of the study site (eg. at home or place of employment) or at scheduled site visits (when applicable), as indicated in the SoA.

In addition to on-site training, instructional materials will be provided to the participant to use as a reference. Participants will contact the site with any questions or concerns they have regarding the self-collection process. Reminder contacts will be made monthly to the participants, to prompt their collection of samples. Receipt of the samples will be tracked in real-time in order to provide feedback to the site. The site will contact and re-train participants who are missing samples or submitting samples which are unacceptable for testing. Detailed instructions will be provided to the site for monitoring self-collection and sample handling.

9.2.1.1.3 CMV PCR Testing

DNA will be extracted from the self-collected urine and saliva samples and assayed for the presence of CMV by a PCR assay. If CMV is detected, distinction between V160 vaccine virus and non-vaccine virus will be performed in a separate PCR assay. Additionally, in order to assess the adequacy of the self-collection, extracted DNA will be assayed for a human housekeeping gene, beta-globin by PCR. Beta-globin testing will stop after 6 months, once samples are demonstrated to be consistently positive. If self-collected samples are not consistently positive or there is concern about declining quality/quantity of DNA isolated, beta-globin testing may be reinstituted.

Refer to Appendix 5 for a summary of the CMV PCR assay.

9.2.1.2 cCMVi

9.2.1.2.1 cCMVi Case Definition

Virologically-confirmed cases of cCMVi will be used to test the efficacy of V160 against cCMVi. A virologically-confirmed case of cCMVi is defined as the occurrence of any of the following events:

1. PCR-positive result by Merck-validated CMV PCR assay in at least 1 urine sample obtained within 21 days of birth from an infant born to a study participant.
    - PCR-negative results by Merck-validated CMV PCR assay in all available urine samples obtained within 21 days of birth from an infant born to a study participant are considered definitive evidence of absence of CMV infection, and therefore constitute a non-case of cCMVi.
2. In the absence of any urine sample obtained within 21 days of birth from an infant born to a study participant and tested by Merck-validated CMV PCR assay, a PCR-positive result by Merck-validated CMV PCR assay in at least 2 saliva samples obtained within 21 days of birth from an infant born to a study participant.
    - The cCMVi case definition based on saliva samples will be applied only in the absence of results from urine samples. PCR-negative results by Merck-validated CMV PCR assay in all available urine samples obtained within 21 days of birth from an infant born to a study participant are considered definitive evidence of absence of CMV infection, and therefore constitute a non-case of cCMVi, regardless of the PCR results in saliva samples.
3. A consensus diagnosis by the Clinical Adjudication Committee (CAC) of a case of cCMVi in the fetus/infant of a study participant based on detection of CMV by PCR or other virologic methods outside the context of the study (ie, based on methods not validated by Merck) from:
    a. saliva, urine, or other body fluid samples (including the neonatal dried blood spot) obtained within 21 days of birth from an infant born to a study participant;

        OR

    b. amniocentesis fluid obtained from a study participant, or samples from still-birth or products of conception obtained from a study participant.

9.2.1.2.2 cCMVi Case Detection

After a pregnancy is reported to the site, the participant will be asked to come to the site for a study visit (referred to as cCMVi Visit 1).

At this visit, the site staff will explain that the participant's pregnancy will be monitored via monthly contacts from the site until the outcome of the pregnancy is established and recorded. Instructions on when to call the site for any changes in pregnancy status will be provided and an explanation and informed consent/assent regarding follow-up of infant/live birth will also be provided.

For the pregnancy follow-up, medical records of the pregnant study participant will be collected and reviewed by the site personnel to:

- confirm date of conception and expected delivery date
- obtain information on the outcome of the pregnancy
- obtain information that could indicate a suspicion/diagnosis of congenital CMV infection and whether the participant had any related interventions
    - Any anti-CMV treatments received by participants during pregnancy or by infants will be collected for the duration of the trial.
- obtain information that could indicate suspected congenital infection in the infant

All relevant medical records should be placed in the participant's trial file. Infant outcomes will be recorded in the eCRF for all pregnant participants within 24 hours of the delivery or as soon as possible. All pregnancies will be followed to outcome. Refer to Section 9.3.6 for additional requirements for pregnancy follow-up and reporting.

For any live birth occurring during the study, the participant will be asked to return to the site with their infant for an additional visit(s) (referred to as cCMVi Visit 2) within 21 days after birth of the infant to collect urine and saliva samples from the infant. During this time, 2 sets of urine and saliva samples at 2 different time points will be collected from the infant to determine the infant's CMV status by PCR testing as described in Section 9.2.1.1.3. Each set of samples can be collected on different days or on the same day, at least 3 hours apart. The saliva sample should not be collected within 2 hours of breastfeeding (if the woman is breastfeeding). Infant sample collection kits will be provided to the sites and site personnel should collect the samples according to the instructions provided. It is critical that both sets of samples are collected within 21 days of the birth of the infant.

If it is not possible for the participant to return to the clinic with their infant to obtain urine and saliva samples, additional efforts will be made to obtain the sample (eg, study personnel may be dispatched to a hospital or the infant's home).

Medical review and adjudication by the CAC will be triggered by suspicion of congenital CMV during pregnancy of a study participant or in the infant.

- The CAC will review all relevant information captured in V160 case report forms.
- Suspicion of congenital CMV during pregnancy of a study participant will be documented and captured in the V160 fetus-infant details (FID) case report form.
- Detection of CMV by PCR or other virologic methods outside the context of the study will also be documented and captured in the FID form.
- Detection of CMV by PCR or other virologic methods outside the context of the study is a necessary requirement for the CAC to arrive at a diagnosis of cCMVi case attributable to the study participant.

Data to be used to determine cases and details regarding the adjudication process will be provided in the Adjudication Charter. Refer to Section 9.2.1.2.1 for the cCMVi case definition and Appendix 1 for information regarding the CAC.

9.2.1.2.3 Health Follow-Up of Infants Diagnosed with cCMVi

Infants who are diagnosed with cCMVi will be followed for up to years 3 after birth to collect hearing, vision, and/ or neurological outcomes as well as any other manifestations of cCMVi. These infant health follow-up visits will be conducted on a yearly basis as outlined in the SoA.

9.2.2 Immunogenicity Assessments

Sera collected from all participants at time points specified in the SoA will be used to measure humoral immune responses to V160 using both viral NAbs and IgG ELISA assays. Blood collected from a subset of subjects at time points specified in the SoA will be used to measure cell-mediated immunity using the CMV ELIPSOT assay.

Blood collection, storage and shipment instructions for serum and blood samples will be provided in the operations/laboratory manual.

Note that all blood samples collected on the day of vaccination will be drawn prior to V160/placebo administration.

Refer to Table 3 for a listing of assays to be done at each visit and volume of blood collected.

9.2.2.1 Viral Neutralizing Antibody Assay

The viral NAb assay measures the functional antibodies blocking viral entry and is considered the primary assay for immunogenicity.

Refer to Appendix 5 for additional details of the assay.

9.2.2.2 IgG ELISA Assay

IgG ELISA measuring total IgG against V160 virus will be validated as a surrogate immunogenicity assay. This assay does not provide a functional read-out of immunogenicity of V160 but is easier to perform. If it is found to correlate with immunogenicity measured by the NAb, it may replace the NAb assay in future studies.

Refer to Appendix 5 for additional details of the assay.

9.2.2.3 CMV IFN-γ ELISPOTs Assay

The CMV interferon gamma (IFN-γ) ELISPOTs (CMV ELISPOT) assay will be used to assess CMI responses by detecting (IFN-γ secreting CMV-specific T cells from PBMCs in response to stimulation of three different CMV antigens (pp65 and IE1 CMV proteins, and purified CMV).

This assay will be performed on a subset of participants as shown in the SoA.

Refer to Appendix 5 for additional details of the assay.

9.3 Adverse Events (AE), Serious Adverse Events (SAE) and Other Reportable Safety Events

The definitions of an adverse event (AE) or serious adverse event (SAE), as well as the method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting AE, SAE and other reportable safety event reports can be found in Appendix 4.

AE, SAEs, and other reportable safety events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator, who is a qualified physician, and any designees are responsible for detecting, assessing, documenting, and reporting events that meet the definition of an AE or SAE as well as other reportable safety events. Investigators remain responsible for following up AE, SAEs and other reportable safety events for outcome according to Section 9.3.3.

9.3.1 Time Period and Frequency for Collecting AE, SAE and Other Reportable Safety Event Information

All AEs, SAEs and other reportable safety events that occur after the consent form is signed but before allocation/randomization must be reported by the investigator if they cause the participant to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of allocation/randomization through 14 days following the first vaccination(s) and from the time of any subsequent vaccination(s) through 14 days thereafter, all AEs, SAEs and other reportable safety events must be reported by the investigator. SAEs (regardless of causality), other reportable safety events (ie, Cancer, Overdose, pregnancy, and infant SAEs) are reportable throughout the duration of an individual’s study participation. Exposure during breast feeding is reportable from Day 1 through Month 7.

Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor if the event is either:

1. A death that occurs prior to the participant completing the trial, but outside the time period specified in the previous paragraph.

or

2. A serious adverse event that is considered by an investigator who is a qualified physician to be vaccine related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.

All initial and follow-up AEs, SAEs and other reportable safety events will be recorded and reported to the sponsor or designee within the timeframes as indicated in Table 4.

Table 4 Reporting Time Periods and Timeframes for Adverse Events and Other Reportable Safety Events

Type of Event		**Reporting Time Period:** **Consent to Randomization/ Allocation**	**Reporting Time Period:** **Randomization/ Allocation through Protocol-Specified Follow-up Period**	**Reporting Time Period:** **After the Protocol Specified Follow-up Period**		**Timeframe to Report Event and Follow-up Information to SPONSOR:**
Non-Serious Adverse Event (NSAE)		Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all from the time of each vaccination through 14 days thereafter.	Not required		Per data entry guidelines
Serious Adverse Event (SAE)		Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all for the duration of an individual's study participation.	Report if: - drug/vaccine related. - any death until participant completion of trial (Follow ongoing to outcome)		Within 24 hours of learning of event
Pregnancy		Report if: - due to intervention - causes exclusion	Report all – Follow to completion/termination and report outcome	Previously reported – Follow to completion/termination; report outcome		Within 24 hours of learning of event
Lactation Exposure		Report if: - due to intervention - causes exclusion	Report all - Day 1 through Month 7	Previously reported – follow to completion		Within 24 hours of learning of event
Cancer		Report if: - due to intervention - causes exclusion	Report all for the duration of an individual's study participation.	Not required		Within 5 calendar days of learning of event
Overdose		Report if: - receiving placebo run-in or other run-in medication	Report all for the duration of an individual's study participation.	Not required		Within 5 calendar days of learning of event

9.3.2 Method of Detecting AE, SAE and Other Reportable Safety Events

Care will be taken not to introduce bias when detecting AE and/or SAE and other reportable safety events. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

9.3.3 Follow-up of AE, SAE and Other Reportable Safety Event Information

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All AE, SAE and other reportable safety events including pregnancy and exposure during breastfeeding, Cancer and Overdose will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 8.3). In addition, the investigator will make every attempt to follow all non-serious AEs that occur in randomized participants for outcome. Further information on follow-up procedures is given in Appendix 4. Although participants are to agree not to become pregnant during the vaccination period through Month 7, if a pregnancy is reported before the last planned V160/placebo dose, the participant will be discontinued from further planned vaccination regimen per exclusion criterion but will not be discontinued from the study. Participants who inadvertently become pregnant before receiving all 3 doses of study vaccine or placebo, and any participant who becomes pregnant after the treatment phase will remain in the trial for follow-up of pregnancy and outcome.

9.3.4 Regulatory Reporting Requirements for SAE

- Prompt notification (within 24 hours) by the investigator to the sponsor of SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations, ie, per ICH Topic E6 (R1) Guidelines for Good Clinical Practice.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAE) from the sponsor will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.3.5 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

There are no disease-related events and/or disease related outcomes not qualifying as AEs or SAEs for this study.

9.3.6 Pregnancy and Exposure During Breastfeeding

Although pregnancy and infant exposure during breastfeeding are not considered adverse events, any pregnancy or infant exposure during breastfeeding in a participant (spontaneously reported to the investigator or their designee) that occurs during the trial are reportable to the Sponsor. A detailed previous pregnancy history will be collected from all randomized participants who report a pregnancy during the study.

All female participants will undergo pregnancy testing based on serum analysis for β-hCG at screening, and urine sample analysis prior to each vaccination. Participants found to be pregnant at Day 1 are not eligible to participate in the trial and participants are instructed to use effective contraception through Month 7, one month after the treatment phase. All pregnancies that occur during the trial are reportable to the Sponsor.

Participants who inadvertently become pregnant before receiving all 3 doses of study vaccine or placebo, and any participant who becomes pregnant after the treatment phase will remain in the trial for follow-up of pregnancy and infant outcome. Infant SAEs for all infants born to participants who received the study vaccine or placebo or who were breastfed during the treatment phase of the trial must be reported for the duration of the trial.

Infant exposure to vaccination during the treatment phase refers to any participant who is breastfeeding an infant and receives a vaccination; all cases of infant exposure must be reported during the trial. Lactation events do not need to be reported if breastfeeding begins after the treatment phase.

All reported pregnancies in randomized participants must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported. Refer to Section 9.2.1.2.3 for additional requirements regarding infant outcomes for confirmed cases of cCMVi.

9.3.7 Events of Clinical Interest (ECIs)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECIs) and must be reported to the Sponsor. There are no ECIs for this study.

9.4 Treatment of Overdose

In this trial, an overdose is defined as receipt of more than 1 dose of study vaccine in a 24-hour period.

Sponsor does not recommend specific treatment for an overdose.

9.5 Safety

Details regarding specific safety procedures/assessments to be performed in this trial are provided below. The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per participant can be found in Table 3.

Planned time points for all safety assessments are provided in the SoA.

9.5.1 Physical Examinations (Full and Targeted Examinations)

A full physical examination should be performed prior to vaccination at Visit 2. It should include vital signs (heart rate, respiratory rate, seated blood pressure, and oral temperature), height, weight, heart and lungs abdomen, as well as head, eyes, ears, nose, and throat (HEENT), skin, lymph nodes, neurological system, and musculoskeletal system. Any clinically significant abnormality should be recorded on the appropriate eCRF. Special attention should be paid to clinical signs related to a previous serious illness.

A directed physical examination should be performed prior to each vaccination at Visit 3 (Month 2) and Visit 4 (Month 6), and at Visit 5 (Month 7). This should include vital signs, heart, lungs, and abdomen.

9.5.2 Vital Signs and Body Temperature

Vital signs and body temperature recordings are part of the physical examination and include oral temperature, heart rate, respiratory rate, and blood pressure. Blood pressure and heart rate measurements will be assessed after the participant has at least 5 minutes of rest in a quiet setting without distractions, and from a sitting position with a completely automated device. Manual techniques will be used only if an automated device is not available.
Oral body temperatures will also be documented by participants using their eVRC during the eVRC-specified postvaccination follow-up period.

9.5.3 Laboratory Procedures and Assessments

Refer to Table 3 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.

- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All protocol-required laboratory assessments, as defined in Table 3, must be conducted in accordance with the laboratory manual and the SoA.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in study participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the appropriate CRF (eg, SLAB).
- For any laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of study treatment, every attempt should be made to perform repeat assessments until the values return to normal or baseline or if a new baseline is established as determined by the investigator.

9.5.3.1 Pregnancy Test

A serum β-hCG pregnancy test (which must be sensitive enough to detect β-hCG at concentrations of 25 IU/L) will be performed for women of childbearing potential during screening.

In addition, a urine β-hCG pregnancy test (which must be sensitive to detect β-hCG at concentrations of 25 IU/L) will be performed on each vaccination day prior to any V160/placebo dose, and at Month 7 (one month after the last dose). A negative urine β-hCG pregnancy test must be documented on the day of vaccination before the administration of V160/placebo. Additional pregnancy tests may be performed at the discretion of the investigator at any time during the study.

From Month 7 through the end of the study, study pregnancy tests will not be performed, however, participants will be asked to report a pregnancy as soon as they become aware of the pregnancy. Refer to Section 9.3.6 for details.

9.5.4 Safety Assessments Using the eVRC

All participants will be observed for 30 minutes after each vaccination for any immediate reactions. If any immediate AEs are observed during this period, the time at which the event occurred within this timeframe, as well as the event itself, any concomitant medications that were administered, and resolution of the event, must be recorded on the appropriate eCRF.

Participants will use the eVRC (Section 9.1.11) to document the following information:

- Oral body temperatures measured from Day 1 (day of vaccination) through Day 14 postdose; any temperature ≥100.4°F (≥38.0ºC) oral or equivalent, will be considered an AE of fever.
- Solicited injection-site AEs (pain/tenderness, erythema/redness, and swelling) from Day 1 through Day 5 postdose;
- Solicited systemic AEs (headache, fatigue, muscle pain, and joint pain) from Day 1 through Day 14 postdose;
- Any other injection-site or systemic AEs from Day 1 through Day 14 postdose; and
- Concomitant medications from Day 1 to Day 14 postdose.

The investigator or delegate will review the data captured on the eVRC with the participant at the subsequent visit after each vaccination (refer to the SoA).

For AEs reported on the eVRC, the investigator will use the information provided by the participant both on the eVRC, and verbally at the time of eVRC review, to apply the appropriate intensity/toxicity grade as described in Appendix 4.

9.6 Pharmacokinetics

PK parameters will not be evaluated in this study.

9.7 Pharmacodynamics

Pharmacodynamic parameters will not be evaluated in this study.

9.8 Biomarkers

Biomarkers are not evaluated in this study.

9.9 Future Biomedical Research Sample Collection

The following specimens are to be obtained as part of Future Biomedical Research:

(and will be stored for future biomedical research)

- DNA for future research
- Leftover main study saliva which test positive from CMV DNA assay stored for future research
- Leftover main study urine which test positive from CMV DNA assay stored for future research
- Leftover main study serum from immunologic testing stored for future research and assay development
- Leftover sub-study PBMC from immunologic testing stored for future research and assay development

9.10 Visit Requirements

Visit requirements are outlined in Section 2 – Schedule of Activities (SoA). Specific procedure-related details are provided above in Section 9 – Study Assessments and Procedures.

10. Statistical Analysis Plan

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, but prior to any unblinding/final database lock, changes made to primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, but prior to unblinding/final database lock, will be documented in a sSAP and referenced in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR.

10.1 Statistical Analysis Plan Summary

Key elements of the statistical analysis plan are summarized below; the comprehensive plan is provided in Sections 10.2 to 10.12. For the purposes of analysis and reporting, the overall study ends when all study efficacy, immunogenicity, and safety data are available and ready for database lock execution.

Study Design Overview	Double-Blind, Randomized, Placebo-Controlled Phase 2b, Multi-center Study to Evaluate the Safety, Tolerability, Efficacy and Immunogenicity of a 2-Dose and a 3-Dose Regimen of V160 (Cytomegalovirus [CMV] Vaccine) in Healthy Seronegative Women, 16 to 35 Years of Age
Treatment Assignment	Participants will be randomized in a 1:1:1 allocation to one of the following 3 treatment arms: • V160 3-dose regimen: will receive 3 doses of V160 administered IM according to a Day 1, Month 2, Month 6 schedule; • V160 2-dose regimen: will receive 2 doses of V160 administered IM according to a Day 1 (V160), Month 2 (placebo), Month 6 (V160) schedule; • Placebo regimen: will receive 3 doses of saline solution placebo administered IM according to a Day 1, Month 2, Month 6 schedule. No stratification based on age, sex or other characteristics will be used in this trial.
Analysis Populations	**Efficacy**: Per-Protocol Efficacy (PPE) **Safety**: All Participants as Treated (APaT)
Primary Endpoint(s)	1. **Efficacy**: number of participants with CMVi in the V160 3-dose regimen and placebo arms. 2. **Safety**: number of participants experiencing solicited injection site AEs within Days 1 through 5 after each vaccination visit; solicited systemic AEs and vaccine-related serious AEs within Days 1 through 14 after each vaccination visit.
Key Secondary Endpoints	**Efficacy**: number of participants with CMVi in the V160 2-dose regimen and placebo arms.

Statistical Methods for Key Efficacy Analyses	The primary and secondary efficacy hypotheses will be evaluated by calculating the efficacy of V160 compared to placebo with respect to the CMVi endpoint. The p-value for testing the hypothesis that VE>0% as well as estimation of the 95% CI of VE will be based on the exact binomial method proposed by Chan and Bohidar [Chan, I. S. F. and Bohidar, N. R. 1998].
Statistical Methods for Key Safety Analyses	There are no *a priori* clinical events identified in this trial as Tier 1 events. Tier 2 events identified in this trial include solicited injection site and systemic AEs; vaccine-related AEs (systemic or serious); any serious AEs (in trial participants or infants born to trial participants); discontinuations due to AEs, AEs by system organ class observed in ≥7 participants in at least one vaccination group and elevated temperatures. Estimates of 95% confidence intervals for between-treatment differences in the percentage of participants with events will be calculated using the Miettinen and Nurminen method [Miettinen, O. and Nurminen, M. 1985].
Interim Analyses	One IA will be performed in this trial relating to the testing of the primary efficacy hypothesis. Results will be reviewed by an eDMC. A summary of the IA is provided below. Further details are provided in Section 10.7. **IA**: • Timing: To be conducted upon accumulation of 15 CMVi cases in the combined V160 3-dose regimen and placebo arms, expected at ≈18 months from trial start. • Testing: The primary efficacy hypothesis will be tested. **Final analysis** • Timing: If the result of testing the primary efficacy hypothesis during the IA was inconclusive, a final analysis will be conducted upon accumulation of 24 CMVi cases in the combined V160 3-dose regimen and placebo arms, expected at ≈3 years from trial start.
Multiplicity	The primary efficacy hypothesis will be tested once at an IA, and if necessary, a second time at a final analysis. The secondary efficacy hypothesis will be tested in one final analysis, without an IA, only if the primary efficacy hypothesis is successfully demonstrated. The overall one-sided trial Type 1 error associated with testing the primary and secondary efficacy hypotheses is ≤2.5% based on a gatekeeping procedure described in Section 10.8.

Sample Size and Power	The power of the decision rules that will be implemented in this trial for testing of the primary efficacy hypothesis is based on a fixed-event design. • Corresponding to the decision rules shown in Figure 2 in Section 10.7, the trial has >90% power to demonstrate that the efficacy of a V160 3-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥80% at an overall one-sided Type 1 error equal to 2.3% (see Table 6). The trial will randomize 2,100 participants equally allocated into one of the 3 treatment arms as described in the **Treatment Assignment** panel of this section. Under study design assumptions described in Section 10.9.1.1, the IA of the primary efficacy hypothesis is expected to be conducted at ≈18 months from trial start; and the final analysis if needed is expected to be conducted at ≈3 years from trial start, as mentioned in the **Interim Analyses** panel of this section. The power of the decision rules that will be implemented in this trial for testing of the secondary efficacy hypothesis is also based on a fixed-event design. • Corresponding to the decision rules shown in Figure 2 in Section 10.7, if the secondary efficacy hypothesis is tested based on a total of ≥15 observed CMVi cases in the combined V160 2-dose and placebo regimens, then the trial has power >85% at an overall one-sided Type 1 error ≤2.5% to demonstrate that the efficacy of a V160 2-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥85% (see Table 7).

10.2 Responsibility for Analyses/In-House Blinding

This trial will be conducted as a double-blind trial under in-house blinding procedures.

The statistical analysis of the data obtained from this trial will be the responsibility of the Clinical Biostatistics department of the Sponsor.

The Clinical Biostatistics department will generate the randomized allocation schedules for assignment of study treatment and selection of immunogenicity sub-study participants as described in Section 7.3. Randomization will be implemented using IRT.

An external statistician and scientific programmer will be identified and will comprise the unblinded team. This team will be unblinded on a participant-level. They will not be involved in any discussions regarding modifications to the protocol, statistical methods, identification of protocol deviations, or data validation efforts at any time during the trial.

This trial has one planned IA, and its key aspects are described in Section 10.7. The eDMC will serve as the primary reviewer of the results of the IA. Treatment-level results of the IA will be provided by the external unblinded statistician to the eDMC. The eDMC will make recommendations relating to trial discontinuation or modification to an Executive Oversight Committee (EOC) (see Appendix 1) of the Sponsor. If the eDMC recommends modifications to the design of the protocol or discontinuation of the trial, the EOC may be unblinded to results at the treatment level in order to act on these recommendations. If necessary, limited additional Sponsor personnel may be unblinded to the treatment level results of the IA in order to act on the recommendations of the eDMC. The extent to which individuals are unblinded with respect to results of IA will be documented by the external unblinded statistician. Additional logistical details will be provided in the eDMC Charter.

Upon reaching the milestone when definitive disposition of the primary and secondary efficacy hypotheses has been reached:

- A database lock will be executed.
- All Sponsor personnel who are members of the study team and the V160 program team will be unblinded and a clinical study report will be written.
- If this milestone is reached at or beyond study Year 3, or if the outcome in this milestone is failure of the test of the primary efficacy hypothesis, then the study investigators and participants will be unblinded upon completion of the clinical study report.

Definitive disposition of the primary and secondary hypotheses is reached when one of the following study outcomes occur:

1. Test of the primary efficacy hypothesis fails.
2. Tests of both the primary and secondary efficacy hypotheses succeed.
3. Test of the primary efficacy hypothesis succeeds but the test of the secondary efficacy hypothesis fails.

If at least the primary efficacy hypothesis is successfully demonstrated prior to reaching study Year 3, the study may continue to follow participants up to study Year 3 in order to continue to accumulate more information relating to vaccine efficacy against CMVi and cCMVi. Under this scenario, study investigators and participants will remain blinded during the continuing follow-up for CMVi and cCMVi information. Upon reaching the third year of the study:

- A database lock will be executed.
- An end-of-study clinical report will be written.
- The study investigators and participants will be unblinded upon completion of the end-of-study clinical report.

For the purpose of external reporting of study results in clinical trial registries (eg, EudraCT and Clinicaltrials.gov), the end-of-study milestone corresponds to the last data generated date, which is the date when all study efficacy, immunogenicity, and safety data are available and ready for database lock execution.

10.3 Hypotheses/Estimation

Objectives and hypotheses of the study are stated in Section 4.

10.4 Analysis Endpoints

The efficacy, immunogenicity, and safety endpoints that will be evaluated are listed below.

10.4.1 Efficacy Endpoints

The efficacy endpoint that will be used to evaluate VE and test the primary and secondary efficacy hypotheses is the number of participants with primary CMVi, described in Section 4 and Section 5.4.1.

CMVi is defined as detection of CMV DNA by PCR in urine or saliva samples.

As stated in Section 4, in the evaluation of the primary and secondary efficacy hypotheses, cases of CMVi will be counted starting after the last dose of the assigned vaccination regimen. As stated in Section 10.5.1, the primary and secondary efficacy hypothesis will be evaluated on the PPE population, where eligibility includes being PCR negative post Day 1 through Month 7. Thus, CMVi cases that will be included in the PPE testing of the primary and secondary efficacy hypothesis are those that occurred after Month 7.

10.4.2 Immunogenicity Endpoints

All immunogenicity endpoints in this study are exploratory endpoints and are listed below. These will be further described and defined in a sSAP that will be written for this study.

- Seroconversion based on the NAb and IgG ELISA assays, defined as transition from CMV seronegative at Day 1 to CMV seropositive at Month 7.
- Seropositivity based on the NAb and IgG ELISA assays at Day 1, Months, 7, 12, 24, and 36.
- GMTs based on the NAb and the IgG ELISA assays at Day 1, Months 7, 12, 24, and 36.
- GMc of SFC/10^6 PBMCs based on the ELISPOT assay Day 1, Months 6, 7, 12, and 24.

10.4.3 Safety Endpoints

As specified in Section 4, Section 5.4.1.3, and Section 10.6.3, the endpoints that will be used to assess the safety and tolerability of 2-dose and 3-dose regimens of V160 include the following:

- Number of participants experiencing solicited injection site AEs within Days 1 through 5 after each vaccination visit; solicited systemic AEs (including tiredness, muscle pain, headache, and joint pain); and vaccine-related systemic AEs, SAEs, and elevated temperatures within Days 1 through 14 after each vaccination visit
- Incidence of any SAE at any time during the study

- Incidence of vaccine-type viral shedding
- Outcomes of pregnancies.

10.5 Analysis Populations

10.5.1 Efficacy Analysis Populations

Per Protocol Efficacy (PPE)

The PPE population will serve as the primary population for the evaluation of VE and for testing the primary and secondary efficacy hypotheses. To be eligible for inclusion in the PPE population, study participants must satisfy the following criteria:

- CMV seronegative at Day 1 and CMV negative by PCR for non-vaccine strain virus from post Day 1 through Month 7
- Have received all 3 injections/vaccinations within the vaccination visit window specified in the SoA (Section 2)
- At any time from Day 1 through Month 7, did not have events, deviations from protocol, or engaged in activities that are deemed by the study Clinical Director to potentially interfere with the evaluation of efficacy or immune response to injections of V160.

Participants whose CMV status by PCR post Day 1 through Month 7 is not evaluable will be ineligible for inclusion in the PPE population. The categories of events, deviations from protocol, or activities that are deemed by the study Clinical Director to potentially interfere with the evaluation of efficacy or immune response to injections of V160 will be identified and documented in a memo to study file prior to the time of the IA for efficacy assessment.

Full Analysis Set (FAS)

The FAS will serve as a supportive analysis population for the evaluation of VE. The FAS population consists of all randomized participants who received at least 1 dose of the correct clinical material corresponding to the treatment regimen the participants were randomized into.

10.5.2 Immunogenicity Analysis Populations

The immunogenicity analysis populations that will be used in exploratory analyses of immunogenicity will be described in the sSAP that will be written for this study.

10.5.3 Safety Analysis Population

All Participants as Treated (APaT)

The APaT population will serve as the primary population for evaluation of safety. The APaT population includes all randomized participants who received at least 1 injection of V160 or placebo, have safety follow-up data, and assigns participants to the treatment arm corresponding to the actual clinical material received. For participants who received injections of V160 or placebo corresponding to a sequence that does not correspond to any of

the protocol-defined treatment arms (ie, cross-treated participants), a safety profile listing will be created separate from the safety reports that will be provided for the protocol-defined treatment arms.

10.6 Statistical Methods

Statistical testing and inference relating to efficacy are described in Section 10.6.1. Statistical methods relating to safety analyses are described in Section 10.6.3. Efficacy results that will be deemed to be statistically significant after consideration of the Type 1 error control strategy are described in Section 10.8. Nominal p-values may be computed for other efficacy analyses, but should be interpreted with caution due to potential issues of multiplicity, sample size, etc. Unless otherwise stated, all statistical tests will be conducted at the α=0.025 (1-sided) level.

10.6.1 Statistical Methods for Efficacy Analyses

To address the primary and secondary efficacy hypotheses, one-sided tests of the null hypothesis that the vaccine efficacy is 0% versus the alternative that vaccine efficacy is >0% will be conducted separately for each of the primary and secondary hypotheses. Each of the primary and secondary efficacy hypotheses will be tested at one-sided α=0.025 level (ie, 2.5% Type 1 error). Vaccine efficacy is defined as:

$$VE = 100\%*\{1-(Rv/Rp)\}$$

where Rv and Rp are the incidence rates CMVi in the V160 dose regimen (3-dose for the primary efficacy hypothesis testing; 2-dose for the secondary efficacy hypothesis testing) and placebo groups, respectively. The incidence rate Rv is defined as Rv = Cv/Tv, where Cv = the count of CMVi cases in the vaccine group and Tv = total person-years of follow-up for efficacy in the vaccine group. The incidence rate Rp is defined similarly. The null hypothesis that vaccine is not efficacious (ie, VE=0%) will be tested by constructing a 100*(1−α)% confidence interval (CI) for VE, denoted as (VE_L, VE_U), based on study data at a particular analysis time point (eg, at IA or at final analysis). The statistical criterion for success with respect to the primary efficacy hypothesis (and likewise for the secondary efficacy hypothesis) will be met if VE_L >0%.

The 100*(1−α) % CI for VE, (VE_L, VE_U), is constructed as follows. Under the assumption that R_v and R_p are the means of independent Poisson processes, and given that there is a total of n = Cv + Cp efficacy endpoint cases observed in the combined V160 dose regimen and placebo groups, then the number of primary efficacy cases Cv in the V160 dose regimen group is distributed as Binomial(n,π), where the binomial probability π is defined as π = TvRv/(TvRv+TpRp). The probability π is a person-years-adjusted estimate of the probability that a particular study participant who became a case of CMVi belongs to the V160 dose regimen group. The lower bound of the 100*(1−α) % exact CI for the probability π is obtained by searching for the proportion π_L such that the probability of observing Cv or more efficacy endpoint cases out of n total efficacy cases is ≤α/2. Similarly, the upper bound of the 100*(1−α)% exact CI for the probability π is obtained by searching for the proportion πU such that the probability of observing Cv or fewer efficacy endpoint cases out of n total

efficacy cases is ≤α/2 [Chan, I. S. F. and Bohidar, N. R. 1998]. VE_L and V_E are then calculated from π_L and π_U as follows:

$$VE_L = 100\%*\{1((U, (1+()\}/(1-\pi_U);$$
$$VE_U = 100\%*\{1((L, (1+()\}/(1-\pi_L);$$

where θ = Tp/Tv is the ratio of the total person-years of follow-up for efficacy in the placebo group over the V160 dose regimen group.

There is only one final analysis of the secondary efficacy endpoint. However, the testing of the secondary efficacy hypothesis may occur at the time of the IA or the final analysis of the primary efficacy hypothesis. The secondary efficacy hypothesis will only be tested if the primary efficacy hypothesis is successfully demonstrated. In addition, the secondary efficacy hypothesis will be tested contingent on accumulation of ≥15 total CMVi cases in the V160 2-dose and placebo regimens (see Figure 2 in Section 10.7, and Section 10.9.1.2). Thus, the possible timing for testing the secondary hypothesis is as follows.

- If the primary hypothesis test is successful at the IA *and* there are ≥15 total CMVi cases in the V160 2-dose and placebo regimens at the time of IA, then the secondary efficacy hypothesis will be tested immediately after the testing of the primary efficacy hypothesis at the time of the IA.
- If the primary hypothesis test is successful at the IA and there are <15 total CMVi cases in the V160 2-dose and placebo regimens at the time of IA, the secondary efficacy hypothesis will not be tested at the IA. In this case, follow-up will continue until at least 15 total CMVi cases in the V160 2-dose and placebo regimens groups are accrued. Upon accrual of ≥15 total CMVi cases in the V160 2-dose and placebo regimens, the secondary efficacy hypothesis will be tested.
- If the primary hypothesis test is not successful at the IA, the secondary efficacy hypothesis will not be tested at the time of the IA.

10.6.2 Statistical Methods for Immunogenicity Analyses

Statistical methods for analysis of exploratory immunogenicity endpoints will be provided in the sSAP that will be written for this study.

10.6.3 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters including AEs and outcomes of pregnancies.

The analysis of safety results will follow a tiered approach (Table 5). The tiers differ with respect to the analyses that will be performed. Safety parameters or adverse events of special interest that are identified *a priori* constitute “Tier 1” safety endpoints that will be subject to inferential testing for statistical significance with p-values and 95% confidence intervals provided for between-group comparisons. Other safety parameters will be considered Tier 2 or Tier 3. Tier 2 parameters will be assessed via point estimates with 95% confidence intervals provided for V160 dose regimen versus placebo comparisons; only point estimates by treatment group are provided for Tier 3 safety parameters. Safety results will be summarized for each vaccination dose and across all three vaccination doses.

There were no SAEs nor vaccine-type viral shedding (which is an event of interest for V160) observed in the V160 Phase I study. While vaccine-type viral shedding is an event of interest that can occur only in V160-vaccinated subjects, barring false-positive tests in placebo recipients, the 0% incidence of vaccine-type viral shedding observed in the Phase I study provides no informed, data-driven, non-zero incidence level around which a safety hypothesis can be formulated and tested. Therefore, there is no *a priori* identified AE or ECI for which a test of hypothesis will be conducted. Thus, no Tier 1 event is identified in this study. Categories of events considered as Tier 2 and Tier 3 in this study are shown in Table 5. In addition, AEs observed in at least 7 participants (representing 1% of N=700 participants per group) in any vaccination group will be categorized as a Tier 2 event. Incidences of the categories of events identified in Table 5 will be reported as percentages, generally calculated as 100% x (number of participants who experienced the event/total number of participants with safety follow-up), except for outcomes of pregnancies, SAEs throughout the study duration, and vaccine-type viral shedding. The 95% CIs will be provided for vaccine versus placebo percentage point differences (percent in V160 dose regimen group minus percent in placebo) will be calculated using the Miettinen and Nurminen method [Miettinen, O. and Nurminen, M. 1985], an unconditional, asymptotic method. No stratification factor will be used in the calculation of 95% CI.

Percentages of outcomes of pregnancies will be generally calculated as 100% x (total number of *specific* pregnancy outcome being reported/total number of *all relevant* pregnancy outcomes). For example, the percent associated with the pregnancy outcome of *live births* will be calculated as 100% x (total number of *live births*/total number of *fetuses/infants with known* outcomes). The numerator and denominator account for pregnancies with multiple fetuses (ie, twins, triplets, etc.) and do not treat such occurrences as a single pregnancy outcome or event. Further details will be provided in the sSAP that will be written for this study.

Vaccine-type viral shedding will be assessed in V160-vaccinated participants. In addition to the analysis strategy shown in Table 5, point and corresponding 95% CI estimates of incidence of vaccine-type viral shedding (number of participants observed with vaccine-type viral shedding/total person-years of follow-up) will be calculated separately for the V160 3-dose, V160 2-dose, and placebo arms. No treatment group comparisons (V160 vs. placebo or V160 3-dose vs. 2-dose arms) of the percent of participants detected with vaccine-type viral shedding will be conducted.

Summaries of SAEs (throughout the study duration) by system organ class will be provided as incidence rates (number of participants [or infants] observed with a specific SAE/total person-years of follow-up) rather than percentages.

Table 5 Analysis Strategy for Safety Parameters

Safety Tier	Safety Endpoint	p-value	95% CI for Comparison of V160 to Placebo	Descriptive Statistics
Tier 2	Solicited injection site AEs[a]		X	X
	Solicited systemic AEs		X	X
	Vaccine-related systemic AEs		X	X
	Any serious AEs (in study participants or infants born to study participants)		X	X[b]
	Vaccine-related serious AEs within Days 1 through 14 after vaccination		X	X
	Discontinuations due to AEs		X	X
	Elevated temperature[c]		X	X
	AEs by SOC observed in ≥7 participants in at least one vaccination group		X	X
Tier 3	AEs by SOC			X
	Outcomes of pregnancies			X
	Vaccine-type viral shedding			X‡

[a] All injection site AEs are considered vaccine-related.
[b] Incidence rates (cases/total person-years follow-up) will be provided instead of percentages.
[c] Defined as ≥38.0ºC [≥100.4°] oral or equivalent
AE = Adverse event; CI = Confidence interval; SOC = System organ class; X = indicated summary statistic will be provided.

10.7 Interim Analyses

10.7.1 Efficacy Interim Analysis

An IA for testing the primary efficacy hypothesis will be conducted upon accrual of a total of 15 CMVi endpoint cases in the combined V160 3-dose and placebo regimens. Possible outcomes of this IA and consequent study pathways are as described below and shown in schematic form in Figure 2.

1. ≥7 of 15 CMVi cases observed in the V160 3-dose arm
   - Study fails to demonstrate the primary efficacy hypothesis at the IA and has no chance of successfully demonstrating the primary efficacy hypothesis in a final analysis when a total of 24 CMVi cases are accrued in the combined V160 3-dose regimen and placebo groups.
   - eDMC may recommend the study to be terminated for futility.
2. (4, 5, or 6) of 15 CMVi cases observed in the V160 3-dose arm.
   - Result of testing of the primary efficacy hypothesis is inconclusive.
   - Study fails to demonstrate the primary efficacy hypothesis at the IA but still has a chance of demonstrating the primary efficacy hypothesis in a final analysis when a

total of 24 CMVi cases are accrued in the combined V160 3-dose regimen and placebo groups.

- Study (which is still blinded) will continue until a total of 24 CMVi cases are accrued in the combined V160 3-dose regimen and placebo groups, at which time, the final testing of the primary efficacy hypothesis will be conducted.

3. ≤3 of 15 CMVi cases observed in the V160 3-dose arm

- Study successfully demonstrates the primary efficacy hypothesis.
- The appropriate timing for the testing the secondary efficacy hypothesis is evaluated (see Section 10.6.1).

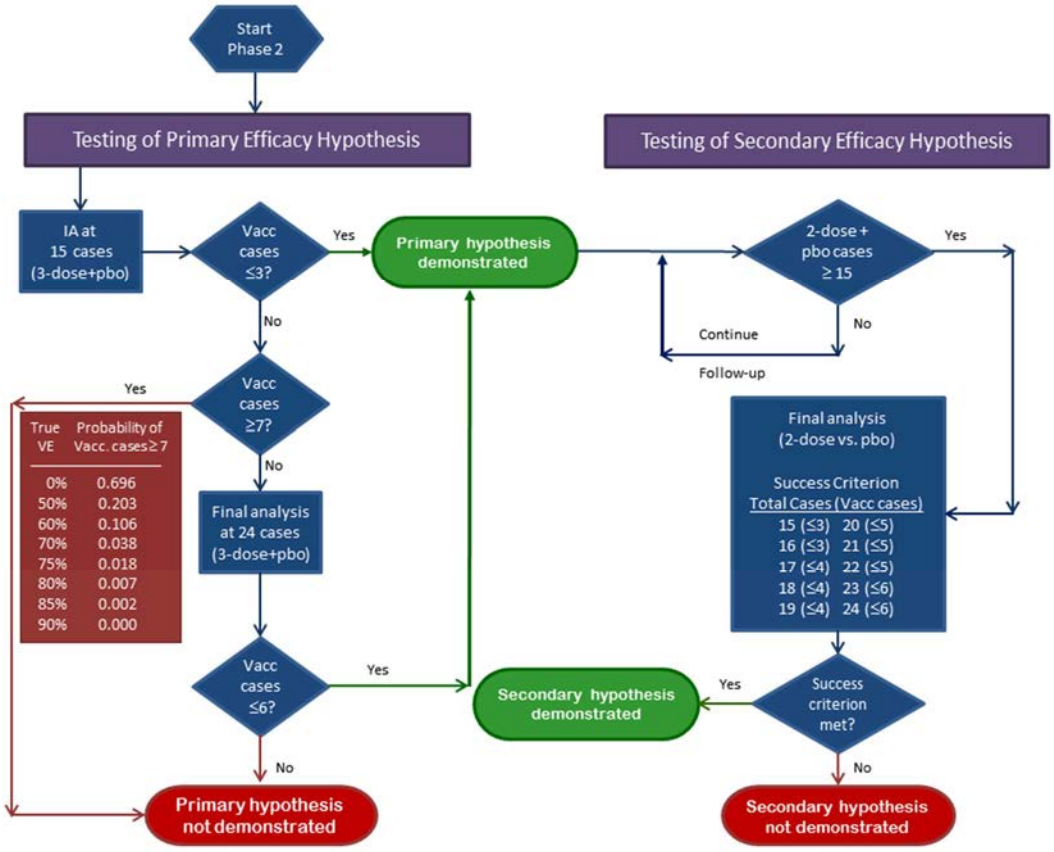


Figure 2 Plan for Testing the Primary and Secondary Efficacy Hypotheses

10.7.2 Safety Interim Analysis

At the time of IA for efficacy, the eDMC will also conduct a review of the cumulative study safety data recorded in the study database upon which the 15 CMVi cases was counted and confirmed to have been accumulated. No hypothesis relating to safety will be tested. Summaries of safety parameters identified in Section 10.6.3 Statistical Methods for Safety Analyses, will be prepared for review by the eDMC.

10.8 Multiplicity

The overall 1-sided Type 1 error in testing the efficacy hypotheses is controlled to not exceed 2.5% based on gatekeeping procedure.

The primary efficacy hypothesis will be tested at an overall (over the IA and final analysis) 1-sided Type 1 error <2.5%, as discussed in Section 10.9.1.1. The secondary efficacy hypothesis will be tested at 1-sided Type 1 error ≤2.5% only if the primary efficacy hypothesis is successfully demonstrated, as discussed in Section 10.9.1.2. The primary efficacy hypothesis is a reasonable gatekeeper to the secondary efficacy hypothesis because if a V160 3-dose regimen with last dose at Month 6 is not efficacious, then a V160 2-dose regimen with last dose also at Month 6 is highly unlikely to be efficacious.

10.9 Sample Size and Power Calculations

10.9.1 Sample Size and Power for Efficacy Analyses

10.9.1.1 Sample Size and Power for Testing the Primary Efficacy Hypothesis

The power corresponding to the decision rule that will be implemented in testing the primary efficacy hypothesis is based on a fixed-event design. For testing the primary efficacy hypothesis, participants will be followed until 15 total CMVi cases in the combined V160 3-dose and placebo regimens have been accumulated, at which point, an IA will be conducted. If the testing of the primary efficacy hypothesis is inconclusive at the IA, follow-up will continue until 24 total CMVi cases have been observed in the combined V160 3-dose and placebo regimens. The decision rules to be implemented that will determine success or failure to demonstrate the primary efficacy hypothesis are as shown in Figure 2 in Section 10.7 and in Table 6. Corresponding to these decision rules, the study has >90% power to demonstrate that the efficacy of a V160 3-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥80% at an overall one-sided Type 1 error equal to 2.3%. The operating characteristics of the decision rules with one IA and a final analysis if necessary are evaluated by a 5 million-replication simulation using SAS v9.4. In the simulation conducted, the underlying probability distribution of accumulation of CMVi cases in the V160 3-dose regimen conditional on the total cases in the combined V160 and placebo regimens is based on an exact method under a large sample Poisson distribution assumption proposed by Chan and Bohidar (1998) [Chan, I. S. F. and Bohidar, N. R. 1998].

This study will randomize 700 participants into the V160 3-dose regimen and 700 into the placebo regimen. The 15 total CMVi cases in the combined V160 3-dose and placebo regimens are projected to be accumulated in ≈18 to 19 months under the following assumptions: 1) all 1,400 participants are enrolled within 18 months; 2) incidence of CMVi in the placebo group is 2% per year; 2) VE=85%, so that the incidence of CMVi in the V160 arm is 0.3% per year; 3) loss-to-follow-up is ≈5% to 10% per year; and 4) participants ineligible for the PPE analysis is ≈2% to 5% of participants randomized. Under the same assumptions, the total of 24 CMVi cases in the combined V160 3-dose and placebo regimens is projected to be accumulated in ≈3 years.

It is possible that at the time when CMVi cases are counted to determine whether a total of 15 cases have been accrued, the actual CMVi cases accumulated will already be greater than 15. In such an eventuality, the IA will be conducted based on the actual number of cases accrued (eg, 16, or 17, or some other count). The operating characteristics shown in Table 6 will be recalculated, which will involve recalculating the total number of events needed at the final analysis in order to maintain an overall (ie, cumulative over the IA and final analysis) 1-sided Type 1 error ≤2.5% and overall power >90% when the underlying vaccine efficacy is ≥80%.

Table 6 Decision Rule and Corresponding Type 1 Error and Power for Testing the Primary Efficacy Hypothesis of VE >0% Against CMVi (3-Dose Regimen vs. Placebo)

Analysis Time Point	Total Cases to Accrue	Stop for Futility (Vacc Cases)	Case Split for Success (Vacc:Pbo)	VE (95% CI)	Cumulative Type I Error	True Vaccine Efficacy					
						70%	75%	80%	85%	90%	95%
						Cumulative Power					
IA	15	≥7	≤3 : ≥12	75.0 (7.4, 95.5)	1.8%	53.2%	64.8%	76.8%	87.8%	95.9%	99.5%
Final†	24	≥7	≤6 : ≥18	66.7 (12.3, 89.2)	2.3%	73.0%	83.7%	92.3%	97.5%	99.6%	100%

† Type I error and power shown are cumulative over the IA and final analysis.

CI = Confidence interval; IA = Interim analysis; VE = Vaccine efficacy.

10.9.1.2 Sample Size and Power for Testing the Secondary Efficacy Hypothesis

The power corresponding to the decision rule that will be implemented in testing the secondary efficacy hypothesis is based on a fixed-event design. The secondary efficacy hypothesis will be tested when a total of ≥15 CMVi cases in the combined V160 2-dose and placebo arms have been observed (see Figure 2 in Section 10.7). When the secondary efficacy hypothesis is tested based on a total of ≥15 CMVi cases, the study has >85% power to demonstrate that the efficacy of a V160 2-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥85% at an overall one-sided Type 1 error ≤2.5% (see Table 7). The calculations are based on an exact method under a large sample Poisson distribution assumption proposed by Chan and Bohidar (1998) [Chan, I. S. F. and Bohidar, N. R. 1998] and carried out using the R software.

This study will randomize 700 participants into the V160 2-dose regimen and 700 into the placebo regimen. Under the same assumptions governing the accumulation of CMVi cases in the V160 3-dose and placebo arms, a total of 15 CMVi cases in the combined V160 2-dose and placebo arms are projected to be accumulated in ≈18 to 19 months; and a total of 24 in ≈3 years.

Table 7 Decision Rule and Corresponding Type 1 Error and Power for Testing the Secondary Efficacy Hypothesis of VE >0% Against CMVi (2-Dose Regimen vs. Placebo)

Analysis Time Point	Total Cases Accrued	Case Split for Success (Vacc:Pbo)	VE (95% CI)	Type I Error	True Vaccine Efficacy					
					70%	75%	80%	85%	90%	95%
					Power					
Final	15	≤3 : ≥12	75.0 (7.4, 95.5)	1.8%	53.2%	64.8%	76.8%	87.8%	95.9%	99.5%
	16	≤3 : ≥13	76.9 (16.0, 95.8)	1.1%	47.7%	59.8%	72.9%	85.4%	94.9%	99.4%
	17	≤4 : ≥13	79.2 (0.4, 92.7)	2.5%	64.7%	75.8%	86.0%	93.9%	98.5%	99.9%
	18	≤4 : ≥14	71.4 (9.0, 93.2)	1.5%	59.6%	71.6%	83.2%	92.5%	98.1%	99.9%
	19	≤4 : ≥15	73.3 (16.3, 93.6)	1.0%	54.5%	67.3%	80.1%	90.9%	97.6%	99.8%
	20	≤5 : ≥15	66.7 (3.5, 90.5)	2.1%	69.3%	80.4%	89.8%	96.2%	99.3%	100%
	21	≤5 : ≥16	68.8 (10.7, 91.0)	1.3%	64.8%	76.9%	87.7%	95.3%	99.1%	100%
	22	≤5 : ≥17	70.6 (16.9, 91.5)	0.8%	60.2%	73.3%	85.3%	94.2%	98.8%	100%
	23	≤6 : ≥17	64.7 (6.2, 88.6)	1.7%	73.2%	84.0%	92.5%	97.7%	99.7%	100%
	24	≤6 : ≥18	66.7 (12.3, 89.2)	1.1%	69.1%	81.1%	90.9%	97.1%	99.6%	100%

CI = Confidence interval; VE = Vaccine efficacy.

10.9.2 Sample Size and Power for Safety Analyses

Testing of hypotheses relating to safety will not be conducted in this study. Thus, power and Type 1 error considerations relating to safety analyses are not applicable in this study.

The probability of observing a specific AE in specific treatment arms of this study depends on the number of vaccinated participants with safety follow-up and the underlying incidence of that specific AE in the study population. In each treatment arm, assuming that all 700 participants randomized into the study will have safety follow-up, then there is a >99% chance of observing at least one specific AE if the incidence of that AE is at least 1 in every 150 participants; >90% chance if the incidence is at least 1 in every 300 participants. If no vaccine-related SAEs are observed among the 700 participants randomized in a specific V160 treatment arm, this study will provide 97.5% confidence that the underlying percentage of participants with vaccine-related SAE is ≤0.5% (1 in every 200 participants) among V160 vaccinated participants in that V160 arm. This is based on calculation of a 1-sided 97.5% upper confidence limit of a binomial proportion using the exact binomial method of Clopper and Pearson [Clopper, C. J. and Pearson, E. S. 1934] and was done using PASS 2008. For a sample size of 700 per treatment arm, Table 8 provides the critical split of participants with AEs between V160 and placebo arms for which it will be possible to state with 95% confidence that the risk difference (defined as percent in V160 dose regimen group minus percent in placebo) excludes 0%. For a specific AE, given a sample size of 700 per arm, the minimum total number of participants with AEs for which it is possible to state with 95% confidence that the risk difference excludes 0% is 5. As stated in Section 10.6.3, AEs observed in at least 7 participants (representing 1% of N=700 participants per group) in any vaccination group will be categorized as a Tier 2 event. The 95% CIs of the percentage point differences were calculated using the Miettinen and Nurminen method [Miettinen, O. and Nurminen, M. 1985].

Table 8 Critical Counts of Participants with Adverse Events in V160 and Placebo Arms Resulting in the Lower Bound of 95% CI Estimate of Risk Difference to be >0%

Observed Count of Participants with Adverse Events			Risk Difference[†] (V160 – Placebo)	
Total	V160 (N=700)	Placebo (N=700)	Percentage Points[‡]	95% CI[§]
5	5	0	0.7%	(0.1%, 1.7%)
6	6	0	0.8%	(0.3%, 1.8%)
7	7	0	1.0%	(0.4%, 2.0%)
8	7	1	0.9%	(0.1%, 1.9%)
9	8	1	1.0%	(0.2%, 2.1%)
10	9	1	1.1%	(0.3%, 2.3%)
11	9	2	1.0%	(0.1%, 2.2%)
12	10	2	1.1%	(0.2%, 2.4%)
13	11	2	1.3%	(0.4%, 2.5%)
14	11	3	1.1%	(0.1%, 2.4%)
15	12	3	1.3%	(0.3%, 2.6%)
16	13	3	1.4%	(0.4%, 2.8%)
17	13	4	1.3%	(0.2%, 2.6%)
18	14	4	1.4%	(0.3%, 2.8%)

Observed Count of Participants with Adverse Events			Risk Difference[†] (V160 – Placebo)	
Total	V160 (N=700)	Placebo (N=700)	Percentage Points[‡]	95% CI[§]
19	15	4	1.6%	(0.4%, 3.0%)
	14	5	1.3%	(0.1%, 2.7%)
20	15	5	1.4%	(0.2%, 2.9%)

[†] Defined as percent of participants in the V160 group with adverse events minus the corresponding percent in the placebo group.
[‡] Calculated as 100%×(V160 – Placebo)÷700.
[§] Calculated based on the Miettinen and Nurminen method.
N = Number of participants randomized.
CI = Confidence interval

10.10 Subgroup Analyses and Effect of Baseline Factors

To determine whether VE is consistent across various subgroups associated with specific baseline participant characteristics, the estimate of the VE (with a nominal 95% CI) for the primary efficacy endpoint (comparing 3-dose regimen versus placebo) and for the secondary efficacy endpoint (comparing 2-dose regimen versus placebo) will be estimated and plotted within each subgroup. Subgroup assessments of VE will be conducted for the following baseline participant characteristics:

- Age category (≤21 vs. >21 years)
- Race (white, non-white)
- Region (US, Ex-US)

For each of the primary and secondary efficacy endpoints, a Forest plot will be produced, which provides the estimated point and 95% CI estimates of VE across the subgroups associated with specific baseline participant characteristics listed above. The consistency of VE across subgroups will be assessed descriptively based on these Forest plots.

10.11 Compliance

10.11.1 Adherence to Scheduled Vaccinations

Compliance to scheduled vaccinations in this study is defined as receipt of all 3 vaccinations of either V160 or placebo. To summarize compliance, the numbers of participants who receive each vaccination will be tabulated. For each of vaccination visits 2 and 3, histograms of the time (in days) of administration of the vaccine or placebo relative to the target vaccination visit will be provided.

10.11.2 Adherence to Scheduled Self-Collection of Urine and Saliva Samples

Accurate and reliable ascertainment of the primary and secondary efficacy endpoints (ie, incidence of CMVi) will depend on compliance of study participants to the self-collection of urine and saliva samples based on the schedule indicated in Section 2. Thus, compliance of

study participants to the self-collection of urine and saliva samples will be assessed as follows.

On a participant-level (say the *i*-th participant among the N=2100 study participants), percent compliance (denoted by P_i) of a study participant to the self-collection of urine and/or saliva samples will be calculated as:

$$P_i = \frac{n_i}{M_i} x100\%$$

where

n_i = number of visits for which either a urine or saliva sample was received from the study participant;

M_i = total number of study visits throughout the study for which a urine or saliva sample is expected to be received from the study participant. This number will vary based on the time when the study participant enters the study and the time when the study ends.

For each of the V160 3-dose, V160 2-dose, and placebo arms, point and 95% CI estimates of the geometric mean of the participant-level percent compliance P_i will be calculated to summarize the vaccination group-level compliance of study participants to the self-collection of urine and saliva samples.

This assessment of compliance of study participants to the self-collection of urine and saliva samples will be conducted in both the PPE and FAS analysis populations.

10.12 Extent of Exposure

As indicated in Section 5.5, participants who will receive an injection of V160 at a vaccination visit will receive a 0.5mL dose containing 100 units of V160. Thus:

- Participants randomized to the 3-dose regimen of V160 are expected to be administered a cumulative total of 1.5 mL containing 300 units of V160 over a 6-months duration;
- Participants randomized to the 2-dose regimen of V160 are expected to be administered a cumulative total of 1.0 mL containing 200 units of V160 over a 6-months duration;
- Participants randomized to placebo are not expected to receive V160.

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12. Appendices

12.1 Appendix 1: Study Governance Considerations

Merck Code of Conduct for Clinical Trials

Merck*
Code of Conduct for Clinical Trials

I. Introduction

A. Purpose

Merck, through its subsidiaries, conducts clinical trials worldwide to evaluate the safety and effectiveness of our products. As such, we are committed to designing, implementing, conducting, analyzing and reporting these trials in compliance with the highest ethical and scientific standards. Protection of participant safety is the overriding concern in the design of clinical trials. In all cases, Merck clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

B. Scope

Such standards shall be endorsed for all clinical interventional investigations sponsored by Merck irrespective of the party (parties) employed for their execution (eg, contract research organizations, collaborative research efforts). This Code is not intended to apply to trials which are observational in nature, or which are retrospective. Further, this Code does not apply to investigator-initiated trials which are not under the control of Merck.

II. Scientific Issues

A. Trial Conduct

1. Trial Design

Except for pilot or estimation trials, clinical trial protocols will be hypothesis-driven to assess safety, efficacy and/or pharmacokinetic or pharmacodynamic indices of Merck or comparator products. Alternatively, Merck may conduct outcomes research trials, trials to assess or validate various endpoint measures, or trials to determine participant preferences, etc.

The design (ie, participant population, duration, statistical power) must be adequate to address the specific purpose of the trial. Research participants must meet protocol entry criteria to be enrolled in the trial.

2. Site Selection

Merck selects investigative sites based on medical expertise, access to appropriate participants, adequacy of facilities and staff, previous performance in Merck trials, as well as budgetary considerations. Prior to trial initiation, sites are evaluated by Merck personnel to assess the ability to successfully conduct the trial.

3. Site Monitoring/Scientific Integrity

Trial sites are monitored to assess compliance with the trial protocol and general principles of Good Clinical Practice. Merck reviews clinical data for accuracy, completeness and consistency. Data are verified versus source documentation according to standard operating procedures. Per Merck policies and procedures, if fraud, misconduct or serious GCP-non-Compliance are suspected, the issues are promptly investigated. When necessary, the clinical site will be closed, the responsible regulatory authorities and ethics review committees notified and data disclosed accordingly.

B. Publication and Authorship

To the extent scientifically appropriate, Merck seeks to publish the results of trials it conducts. Some early phase or pilot trials are intended to be hypothesis-generating rather than hypothesis testing. In such cases, publication of results may not be appropriate since the trial may be underpowered and the analyses complicated by statistical issues of multiplicity.

Merck's policy on authorship is consistent with the requirements outlined in the ICH-Good Clinical Practice guidelines. In summary, authorship should reflect significant contribution to the design and conduct of the trial, performance or interpretation of the analysis, and/or writing of the manuscript. All named authors must be able to defend the trial results and conclusions. Merck funding of a trial will be acknowledged in publications.

III. Participant Protection

A. IRB/IEC review

All clinical trials will be reviewed and approved by an independent IRB/IEC before being initiated at each site. Significant changes or revisions to the protocol will be approved by the IRB/IEC prior to implementation, except that changes required urgently to protect participant safety and well-being may be enacted in anticipation of IRB/IEC approval. For each site, the IRB/IEC and Merck will approve the participant informed consent form.

B. Safety

The guiding principle in decision-making in clinical trials is that participant welfare is of primary importance. Potential participants will be informed of the risks and benefits of, as well as alternatives to, trial participation. At a minimum, trial designs will take into account the local standard of care. Participants are never denied access to appropriate medical care based on participation in a Merck clinical trial.

All participation in Merck clinical trials is voluntary. Participants are enrolled only after providing informed consent for participation. Participants may withdraw from a Merck trial at any time, without any influence on their access to, or receipt of, medical care that may otherwise be available to them.

C. Confidentiality

Merck is committed to safeguarding participant confidentiality, to the greatest extent possible. Unless required by law, only the investigator, sponsor (or representative) and/or regulatory authorities will have access to confidential medical records that might identify the research participant by name.

D. Genomic Research

Genomic Research will only be conducted in accordance with informed consent and/or as specifically authorized by an Ethics Committee.

IV. Financial Considerations

A. Payments to Investigators

Clinical trials are time- and labor-intensive. It is Merck's policy to compensate investigators (or the sponsoring institution) in a fair manner for the work performed in support of Merck trials. Merck does not pay incentives to enroll participants in its trials. However, when enrollment is particularly challenging, additional payments may be made to compensate for the time spent in extra recruiting efforts.

Merck does not pay for participant referrals. However, Merck may compensate referring physicians for time spent on chart review to identify potentially eligible participants.

B. Clinical Research Funding

Informed consent forms will disclose that the trial is sponsored by Merck, and that the investigator or sponsoring institution is being paid or provided a grant for performing the trial. However, the local IRB/IEC may wish to alter the wording of the disclosure statement to be consistent with financial practices at that institution. As noted above, publications resulting from Merck trials will indicate Merck as a source of funding.

C. Funding for Travel and Other Requests

Funding of travel by investigators and support staff (eg, to scientific meetings, investigator meetings, etc.) will be consistent with local guidelines and practices including, in the U.S., those established by the American Medical Association (AMA).

V. Investigator Commitment

Investigators will be expected to review Merck's Code of Conduct as an appendix to the trial protocol, and in signing the protocol, agree to support these ethical and scientific standards.

* In this document, "Merck" refers to Merck Sharp & Dohme Corp. and Schering Corporation, each of which is a subsidiary of Merck & Co., Inc. Merck is known as MSD outside of the United States and Canada. As warranted by context, Merck also includes affiliates and subsidiaries of Merck & Co., Inc."

Financial Disclosure

Financial Disclosure requirements are outlined in the US Food and Drug Administration Regulations, Financial Disclosure by Clinical Investigators (21 CFR Part 54). It is the Sponsor's responsibility to determine, based on these regulations, whether a request for Financial Disclosure information is required. It is the investigator's/subinvestigator's responsibility to comply with any such request.

The investigator/subinvestigator(s) agree, if requested by the Sponsor in accordance with 21 CFR Part 54, to provide his/her financial interests in and/or arrangements with the Sponsor to allow for the submission of complete and accurate certification and disclosure statements. The investigator/subinvestigator(s) further agree to provide this information on a Certification/Disclosure Form, commonly known as a financial disclosure form, provided by the Sponsor. The investigator/subinvestigator(s) also consent to the transmission of this information to the Sponsor in the United States for these purposes. This may involve the transmission of information to countries that do not have laws protecting personal data.

Data Protection

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Confidentiality of Data

By signing this protocol, the investigator affirms to the Sponsor that information furnished to the investigator by the Sponsor will be maintained in confidence, and such information will be divulged to the institutional review board, ethics review committee (IRB/IEC) or similar or expert committee; affiliated institution and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this trial will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

Confidentiality of Participant Records

By signing this protocol, the investigator agrees that the Sponsor (or Sponsor representative), IRB/IEC, or regulatory authority representatives may consult and/or copy trial documents in order to verify worksheet/case report form data. By signing the consent form, the participant agrees to this process. If trial documents will be photocopied during the process of verifying

worksheet/case report form information, the participant will be identified by unique code only; full names/initials will be masked prior to transmission to the Sponsor.

By signing this protocol, the investigator agrees to treat all participant data used and disclosed in connection with this trial in accordance with all applicable privacy laws, rules and regulations.

Confidentiality of IRB/IEC Information

The Sponsor is required to record the name and address of each IRB/IEC that reviews and approves this trial. The Sponsor is also required to document that each IRB/IEC meets regulatory and ICH GCP requirements by requesting and maintaining records of the names and qualifications of the IRB/IEC members and to make these records available for regulatory agency review upon request by those agencies.

Committees Structure

Scientific Advisory Committee

This trial was developed in collaboration with a Scientific Advisory Committee (SAC). The SAC comprises both Sponsor and non-Sponsor scientific experts who provide input with respect to trial design, interpretation of trial results and subsequent peer-reviewed scientific publications.

Executive Oversight Committee

The Executive Oversight Committee (EOC) comprises members of Sponsor Senior Management. The EOC will receive and decide upon any recommendations made by the Data Monitoring Committee (DMC) regarding the trial.

Data Monitoring Committee

To supplement the routine trial monitoring outlined in this protocol, an external Data Monitoring Committee (DMC) will monitor the interim data from this trial. The voting members of the committee are external to the Sponsor. The members of the DMC must not be involved with the trial in any other way (eg, they cannot be trial investigators) and must have no competing interests that could affect their roles with respect to the trial.

The DMC will make recommendations to the EOC regarding steps to ensure both participant safety and the continued ethical integrity of the trial. Also, the DMC will review interim trial results, consider the overall risk and benefit to trial participants (see Section 10.7 - Interim Analyses) and recommend to the EOC if the trial should continue in accordance with the protocol.

Specific details regarding composition, responsibilities, and governance, including the roles and responsibilities of the various members and the Sponsor protocol team; meeting facilitation; the trial governance structure; and requirements for and proper documentation of DMC reports, minutes, and recommendations will be described in the DMC charter that is reviewed and approved by all the DMC members.

Clinical Adjudication Committee

A Clinical Adjudication Committee (CAC) will evaluate the potential cCMVi cases for the purposes of confirming them according to the criteria in Section 9.2.1.2.1 – cCMVi Case Definition. Cases confirmed as cCMVi by the CAC will be used in the analysis of the cCMVi endpoint described in the sSAP.

All personnel involved in the adjudication process will remain blinded to study treatment allocation throughout the trial.

Publication Policy

The results of this study may be published or presented at scientific meetings. The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

If publication activity is not directed by the sponsor, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Amendments Act (FDAAA) of 2007 and the European Medicines Agency (EMA) clinical trial Directive 2001/20/EC, the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to http://www.clinicaltrials.gov, www.clinicaltrialsregister.eu or other local registries. Merck, as Sponsor of this trial, will review this protocol and submit the information necessary to fulfill these requirements. Merck entries are not limited to FDAAA or the EMA clinical trial directive mandated trials. Information posted will allow participants to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

By signing this protocol, the investigator acknowledges that the statutory obligations under FDAAA, the EMA clinical trials directive or other locally mandated registries are that of the Sponsor and agrees not to submit any information about this trial or its results to those registries.

Compliance with Law, Audit and Debarment

By signing this protocol, the investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice (eg, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated

Guideline and other generally accepted standards of good clinical practice); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical trial.

The Code of Conduct, a collection of goals and considerations that govern the ethical and scientific conduct of clinical investigations sponsored by Merck, is provided in this appendix under the Merck Code of Conduct for Clinical Trials.

The investigator agrees not to seek reimbursement from participants, their insurance providers or from government programs for procedures included as part of the trial reimbursed to the investigator by the Sponsor.

The investigator will promptly inform the Sponsor of any regulatory authority inspection conducted for this trial.

The Investigator agrees to provide the Sponsor with relevant information from inspection observations/findings to allow the Sponsor to assist in responding to any citations resulting from regulatory authority inspection, and will provide the Sponsor with a copy of the proposed response for consultation before submission to the regulatory authority.

Persons debarred from conducting or working on clinical trials by any court or regulatory authority will not be allowed to conduct or work on this Sponsor's trials. The investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the trial is debarred or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened.

Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator or qualified designee is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

Detailed information regarding Data Management procedures for this protocol will be provided separately.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Trial documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested by the Sponsor or regulatory authority as a result of an audit or inspection to cure deficiencies in the trial documentation and worksheets/case report forms.

The sponsor or designee is responsible for the data management of this study including quality checking of the data.

Study monitors will perform ongoing source data review and verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Study and Site Closure

The sponsor or its designee may stop the study or study site participation in the study for medical, safety, regulatory, administrative, or other reasons consistent with applicable laws, regulations, and GCP.

In the event the Sponsor prematurely terminates a particular trial site, the Sponsor will promptly notify that trial site's IRB/IEC.

12.2 Appendix 2: Collection and Management of Specimens for Future Biomedical Research

1. Definitions

a. Biomarker: A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition.[1]

b. Pharmacogenomics: The investigation of variations of DNA and RNA characteristics as related to drug/vaccine response.[2]

c. Pharmacogenetics: A subset of pharmacogenomics, pharmacogenetics is the influence of variations in DNA sequence on drug/vaccine response.[2]

d. DNA: Deoxyribonucleic acid.

e. RNA: Ribonucleic acid.

2. Scope of Future Biomedical Research

The specimens consented and/or collected in this trial as outlined in Section 9.9 – Future Biomedical Research Sample Collection9.9 – Future Biomedical Research Sample Collection will be used in various experiments to understand:

- The biology of how drugs/vaccines work
- Biomarkers responsible for how a drug/vaccine enters and is removed by the body
- Other pathways drugs/vaccines may interact with
- The biology of disease

The specimen(s) may be used for future assay development and/or drug/vaccine development.

It is now well recognized that information obtained from studying and testing clinical specimens offers unique opportunities to enhance our understanding of how individuals respond to drugs/vaccines, enhance our understanding of human disease and ultimately improve public health through development of novel treatments targeted to populations with the greatest need. All specimens will be used by the Sponsor or those working for or with the Sponsor.

3. Summary of Procedures for Future Biomedical Research

a. Participants for Enrollment

All participants enrolled in the clinical trial will be considered for enrollment in the Future Biomedical Research sub-trial.

b. Informed Consent

Informed consent for specimens (ie, DNA, RNA, protein, etc.) will be obtained during screening for protocol enrollment from all participants or legal guardians, at a trial visit by the investigator or his or her designate. Informed consent for Future Biomedical Research should be presented to the participants on the visit designated in the SoA. If delayed, present consent at next possible Participant Visit. Consent

forms signed by the participant will be kept at the clinical trial site under secure storage for regulatory reasons.

A template of each trial site's approved informed consent will be stored in the Sponsor's clinical document repository.

c. eCRF Documentation for Future Biomedical Research Specimens

Documentation of participant consent for Future Biomedical Research will be captured in the electronic Case Report Forms (eCRFs). Any specimens for which such an informed consent cannot be verified will be destroyed.

d. Future Biomedical Research Specimen(s)

Collection of specimens for Future Biomedical Research will be performed as outlined in the SoA. In general, if additional blood specimens are being collected for Future Biomedical Research, these will usually be obtained at a time when the participant is having blood drawn for other trial purposes.

4. Confidential Participant Information for Future Biomedical Research

In order to optimize the research that can be conducted with Future Biomedical Research specimens, it is critical to link participant' clinical information with future test results. In fact little or no research can be conducted without connecting the clinical trial data to the specimen. The clinical data allow specific analyses to be conducted. Knowing participant characteristics like gender, age, medical history and treatment outcomes are critical to understanding clinical context of analytical results.

To maintain privacy of information collected from specimens obtained for Future Biomedical Research, the Sponsor has developed secure policies and procedures. All specimens will be single-coded per ICH E15 guidelines as described below.

At the clinical trial site, unique codes will be placed on the Future Biomedical Research specimens. This code is a random number which does not contain any personally identifying information embedded within it. The link (or key) between participant identifiers and this unique code will be held at the trial site. No personal identifiers will appear on the specimen tube.

5. Biorepository Specimen Usage

Specimens obtained for the Sponsor will be used for analyses using good scientific practices. Analyses utilizing the Future Biomedical Research specimens may be performed by the Sponsor, or an additional third party (eg, a university investigator) designated by the Sponsor. The investigator conducting the analysis will follow the Sponsor's privacy and confidentiality requirements. Any contracted third party analyses will conform to the specific scope of analysis outlined in this sub-trial. Future Biomedical Research specimens remaining with the third party after specific analysis is performed will be reported to the Sponsor.

6. Withdrawal From Future Biomedical Research

Participants may withdraw their consent for Future Biomedical Research and ask that their biospecimens not be used for Future Biomedical Research. Participants may withdraw consent at any time by contacting the principal investigator for the main trial. If

medical records for the main trial are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com). Subsequently, the participant's specimens will be flagged in the biorepository and restricted to main study use only. If specimens were collected from study participants specifically for Future Biomedical Research, these specimens will be removed from the biorepository and destroyed. Documentation will be sent to the investigator confirming withdrawal and/or destruction, if applicable. It is the responsibility of the investigator to inform the participant of completion of the withdrawal and/or destruction, if applicable. Any analyses in progress at the time of request for withdrawal/destruction or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (eg, if the investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the participant's personal information and their specimens. In this situation, the request for withdrawal of consent and/or destruction cannot be processed.

7. Retention of Specimens

Future Biomedical Research specimens will be stored in the biorepository for potential analysis for up to 20 years from the end of the main study. Specimens may be stored for longer if a regulatory or governmental authority has active questions that are being answered. In this special circumstance, specimens will be stored until these questions have been adequately addressed.

Specimens from the trial site will be shipped to a central laboratory and then shipped to the Sponsor-designated biorepository. If a central laboratory is not utilized in a particular trial, the trial site will ship directly to the Sponsor-designated biorepository. The specimens will be stored under strict supervision in a limited access facility which operates to assure the integrity of the specimens. Specimens will be destroyed according to Sponsor policies and procedures and this destruction will be documented in the biorepository database.

8. Data Security

Databases containing specimen information and test results are accessible only to the authorized Sponsor representatives and the designated trial administrator research personnel and/or collaborators. Database user authentication is highly secure, and is accomplished using network security policies and practices based on international standards to protect against unauthorized access.

9. Reporting of Future Biomedical Research Data to Participants

No information obtained from exploratory laboratory studies will be reported to the participant, family, or physicians. Principle reasons not to inform or return results to the participant include: Lack of relevance to participant health, limitations of predictive capability, and concerns regarding misinterpretation.

If important research findings are discovered, the Sponsor may publish results, present results in national meetings, and make results accessible on a public website in order to rapidly report this information to doctors and participants. Participants will not be identified by name in any published reports about this study or in any other scientific publication or presentation.

10. Future Biomedical Research Study Population

Every effort will be made to recruit all participants diagnosed and treated on Sponsor clinical trials for Future Biomedical Research.

11. Risks Versus Benefits of Future Biomedical Research

For future biomedical research, risks to the participant have been minimized and are described in the Future Biomedical Research informed consent.

The Sponsor has developed strict security, policies and procedures to address participant data privacy concerns. Data privacy risks are largely limited to rare situations involving possible breach of confidentiality. In this highly unlikely situation there is risk that the information, like all medical information, may be misused.

12. Questions

Any questions related to the future biomedical research should be e-mailed directly to clinical.specimen.management@merck.com.

13. References

1. National Cancer Institute: http://www.cancer.gov/dictionary/?searchTxt=biomarker
2. International Conference on Harmonization: DEFINITIONS FOR GENOMIC BIOMARKERS, PHARMACOGENOMICS, PHARMACOGENETICS, GENOMIC DATA AND SAMPLE CODING CATEGORIES - E15; http://www.ich.org/LOB/media/MEDIA3383.pdf
3. Industry Pharmacogenomics Working Group. Understanding the Intent, Scope and Public Health Benefits of Exploratory Biomarker Research: A Guide for IRBs/IECs and Investigational Site Staff. Available at http://i-pwg.org/
4. Industry Pharmacogenomics Working Group. Pharmacogenomics Informational Brochure for IRBs/IECs and Investigational Site Staff. Available at http://i-pwg.org/

12.3 Appendix 3: Contraceptive Guidance and Pregnancy Testing

Only women of childbearing potential (WOCBP) 16 to 35 years of age are to be enrolled in this study.

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are **NOT** considered WOCBP, and therefore will NOT be enrolled:

- Premenopausal female with 1 or more of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
  - Documented bilateral tubal ligation

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 9 during the protocol-defined time frame in Section 6.1.

Table 9 Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
• Combined (estrogen- and progestogen- containing) hormonal contraception [b] o Oral o Intravaginal o Transdermal o Injectable
• Progestogen-only hormonal contraception [b] o Oral o Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
• Progestogen- only contraceptive implant [b] • Intrauterine hormone-releasing system (IUS) • Intrauterine device (IUD)
• Vasectomized partner A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
• Sexual abstinence Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. As WOCBP are the target population of this study and pregnancy is permissible after the treatment phase, permanent contraception methods at study entry are not allowed. [a] Typical use failure rates are lower than perfect-use failure rates (ie, when used consistently and correctly). [b] If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

Pregnancy Testing

Women of childbearing potential are to be included after a negative highly sensitive urine or serum pregnancy test and in accordance with local requirements. This test should be repeated a maximum of 24-hours before the first dose/vaccination.

12.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
● An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study treatment, whether or not considered related to the study treatment. ● NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study treatment. ● NOTE: for purposes of AE definition, study treatment (also referred to as Sponsor's product) includes any pharmaceutical product, biological product, vaccine, device, diagnostic agent or protocol specified procedure whether investigational (including placebo or active comparator product) or marketed, manufactured by, licensed by, provided by or distributed by the sponsor for human use in this study.

Events <u>Meeting</u> the AE Definition
● Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, or are considered clinically significant in the medical and scientific judgment of the investigator. ● Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. ● New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. ● Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. ● Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. ● For all reports of overdose (whether accidental or intentional) with an associated adverse event, the AE term should reflect the clinical symptoms or abnormal test result. An overdose without any associated clinical symptoms or abnormal laboratory results is reported using the terminology "accidental or intentional overdose without adverse effect." ● Any new cancer or progression of existing cancer.

Events NOT Meeting the AE Definition
● Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. ● Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). ● Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen. ● Surgery planned prior to informed consent to treat a pre-existing condition that has not worsened. ● Refer to section 9.3.5 for protocol specific exceptions

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening ● The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization ● Hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.
d. Results in persistent or significant disability/incapacity ● The term disability means a substantial disruption of a person's ability to conduct normal life functions. ● This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

- in offspring of participant taking the product regardless of time to diagnosis

f. Other important medical events:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Additional Events Reported

Additional Events which require reporting
In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor. • Is a cancer; • Is associated with an overdose

Recording AE and SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event. • The investigator will record all relevant AE/SAE information on the Adverse Event case report forms/worksheets at each examination. • It is **not** acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the AE CRF page. • There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to the Sponsor. • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

- An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.
- The investigator will make an assessment of intensity for each AE and SAE (and other reportable safety event) reported during the study and assign it to 1 of the following categories:
  - Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities. (for pediatric trials, awareness of symptoms, but easily tolerated)
  - Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities. (for pediatric trials, definitely acting like something is wrong)
  - Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe (for pediatric trials, extremely distressed or unable to do usual activities).
  - Injection site erythema/redness or induration/swelling from the day of vaccination through Day 5 postvaccination will be evaluated by maximum size.
  - The investigator will assess toxicity for each AE and SAE (and other reportable event) reported during the study. A toxicity grade will be assigned to injection-site AEs, specific systemic AEs, other systemic AEs, and vital sign (temperature) as shown in the following tables. The toxicity grading scales used in this study are adapted from the "FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2007."

Injection Site Reactions Intensity/Toxicity Grading Scale

Injection Site Reaction to V160/Placebo[a]	Grade 1	Grade 2	Grade 3	Grade 4
Injection-site AEs occurring Days 1 through 5 following receipt of V160/placebo				
Pain/Tenderness	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Erythema/Redness	Size measured as B	Size measured as C or D	Size measured as E→	Necrosis or exfoliative dermatitis or results in ER visit or hospitalization
Induration/Swelling	Size measured as B	Size measured as C or D	Size measured as E→	Necrosis or ER visit or hospitalization

Other	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Any injection-site reaction that begins ≥6 days after receipt of V160/placebo				
Pain/tenderness Erythema/Redness Induration/Swelling Other	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization

Abbreviations: AE = adverse event; ER = emergency room; eVRC = electronic Vaccine Report Card; SAE = serious adverse event

[a] Based upon information provided by the patient on the eVRC and verbally during VRC review. Erythema/Redness/Induration and Swelling are specific injection-site AEs with size designations of letters A through E→, based upon a graphic in the eVRC. Size A is not assigned a toxicity grade; however, injection-site AEs that measure size A should be reported as adverse experiences. If the participant has an ER visit or is hospitalized for any injection-site AE, that AE is to be assigned a toxicity grade of 4, regardless of the size measured.

Specific Systemic AE Intensity/Toxicity Grading Scale

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Abbreviations: ER = emergency room

Other Systemic AEs Intensity/Toxicity Grading Scale

Systemic Illness[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[b]
Illness or clinical AE (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and required medical intervention	ER visit or hospitalization

Abbreviations: ER = emergency room; eVRC = electronic Vaccine Report Card; SAE = serious adverse event
[a] Based upon information provided by the patient on the eVRC and verbally during the eVRC review during the primary safety follow-up period. For SAEs reported beyond the primary safety follow-up period, grading will be based upon the initial report and/or follow-up of the event.
[b] AEs resulting in death will be assessed as Grade 4

Vital Sign (Temperature) Toxicity Grading Scale

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) [b]	38.0° to 38.4°	38.5° to 38.9°	39.0° to 40.0°	>40.0°
(°F) [b]	100.4° to 101.1°	101.2° to 102.0°	102.1° to 104.0°	>104.0°

[a] Participant should be at rest for all vital sign requirements
[b] Oral temperature; no recent hot or cold beverages or smoking

Assessment of Causality

- Did the Sponsor's product cause the adverse event?
  - The determination of the likelihood that the Sponsor's product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test product and the adverse event based upon the available information
  - **The following components are to be used to assess the relationship between the Sponsor's product and the AE;** the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Sponsor's product caused the adverse event:
    - **Exposure:** Is there evidence that the participant was actually exposed to the Sponsor's product such as: reliable history, acceptable compliance assessment (diary, etc.), seroconversion or identification of vaccine virus in bodily specimen?
    - **Time Course:** Did the AE follow in a reasonable temporal sequence from administration of the Sponsor's product? Is the time of onset of the AE compatible with a vaccine-induced effect?
    - **Likely Cause:** Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors
    - **Rechallenge:** Was the participant re-exposed to the Sponsor's product in this trial?
      - If yes, did the AE recur or worsen?
        - If yes, this is a positive rechallenge.
      - If no, this is a negative rechallenge.

      (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose vaccine trial); or (3) Sponsor's product(s) is/are used only one time.)

NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE SPONSOR'S PRODUCT, OR IF RE-EXPOSURE TO THE SPONSOR'S PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE PARTICIPANT THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR CLINICAL DIRECTOR, AND IF REQUIRED, THE INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE.

- **Consistency with Study treatment Profile:** Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Sponsor's product or drug class pharmacology or toxicology?

- The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.
- Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Sponsor's product relationship).
  - Yes, there is a reasonable possibility of Sponsor's product relationship: There is evidence of exposure to the Sponsor's product. The temporal sequence of the AE onset relative to the administration of the Sponsor's product is reasonable. The AE is more likely explained by the Sponsor's product than by another cause.
  - No, there is not a reasonable possibility of Sponsor's product relationship: Participant did not receive the Sponsor's product OR temporal sequence of the AE onset relative to administration of the Sponsor's product is not reasonable OR the AE is more likely explained by another cause than the Sponsor’s product. (Also entered for a participant with overdose without an associated AE.)
- For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor.
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements

Follow-up of AE and SAE
● The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. ● New or updated information will be recorded in the CRF. ● The investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

Reporting of AE, SAE, and Other Reportable Safety Events to the Sponsor

AE, SAE, and Other Reportable Safety Event Reporting to Sponsor via Electronic Data Collection Tool

● The primary mechanism for reporting to the Sponsor will be the electronic data collection (EDC) tool.
  ● Electronic reporting procedures can be found in the EDC data entry guidelines (or equivalent).
  ● If the electronic system is unavailable for more than 24 hours, then the site will use the paper AE Reporting form.
  ● Reference section 9.3.1 – Time Period and Frequency for Collecting AE and SAE and Other Reportable Safety Event Information for reporting time requirements
● The site will enter the SAE data into the electronic system as soon as it becomes available.
● After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
● If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form or by telephone (see next section).
● Contacts for SAE reporting can be found in the Investigator Trial File Binder (or equivalent).

SAE Reporting to the Sponsor via Paper CRF
● If the electronic data collection tool is not operational, facsimile transmission or secure e-mail of the SAE paper CRF is the preferred method to transmit this information to the Sponsor. ● In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. ● Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. ● Contacts and instructions for SAE reporting and paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

12.5 Appendix 5: Laboratory Assays

Safety laboratory assessments (ie, blood chemistry, hematology and urinalysis) are not planned for this study.

A serum pregnancy test will be performed at screening as indicated in the SoA (protocol-specific requirements for inclusion or exclusion of participants are detailed in Sections 6.1 and 6.2) and a urine pregnancy test will be performed prior to each vaccine dose and one month after the last dose. Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations. Investigators must document their review of any laboratory safety report.

Any laboratory results that could unblind the study will not be reported to investigative sites or other blinded personnel (until the study has been unblinded).

Efficacy and Immunogenicity Laboratory Assays

CMV PCR Assays

The purpose of the CMV polymerase chain reaction (PCR) assay is to detect CMV both non-vaccine type and vaccine-type strain deoxyribonucleic acid DNA in specimens obtained from participants.

The specificity of the PCR assay has been evaluated against a panel of viral and non-viral targets to confirm 1) specific amplification of CMV and 2) absence of cross-reactivity to the other microorganisms tested. PCR sensitivity for urine and saliva detection will be determined prior to initiation of sample testing, with a target goal to achieve sensitivity of at least 200 IU/mL for both sample types. This will help assess whether V160 is able to establish latency, shed, and potentially serve as a source of transmission to close contacts (unexpected).

This is the primary assay for the detection of vaccine-type viral shedding in clinical samples. The assay is planned to be performed by an outside vendor on behalf of Merck Research Laboratories.

The assay uses virus-specific primers and probes to detect and discriminate among wild type CMV and those of the vaccine strain-specific construct.

The CMV PCR assay system is designed as a two-part assay to 1) detect and quantify low levels of CMV DNA and 2) discriminate Merck vaccine virus from non-vaccine type viral strains.

Viral DNA from urine and saliva clinical samples will be extracted using a commercially available DNA purification kit and initially PCR tested using UL54 and UL55 as the viral DNA target in a quantitative PCR (qPCR) assay to determine the presence and quantity of any CMV.

Samples identified as positive for CMV DNA using the initial assay will then be tested using a multiplex CMV real time qPCR (RT-PCR) assay to detect 3 specific targets (US28/29 junction region; loxP; and UL51-FKBP fusion) for the discrimination of vaccine-derived viral DNA from wild type viral DNA. In the vaccine discrimination assay, the US28/29 junction region is found in both vaccine and wild type strains. The loxP sequence is a bacterial recombination sequence and is only found in bacterial artificial chromosome

(BAC)- derived CMV strains (ie, the vaccine strain). The UL51-FKBP target is a genetically engineered gene fusion specific to the Merck vaccine strain.

For both components of the CMV PCR assay system, analysis of the raw fluorescence data is generated and captured using RT-PCR instrumentation.

For CMV qPCR, the number of DNA copies in each sample is quantified compared to a standard curve which is calibrated to an international standard.

For RT-PCR the qualitative assessment of the presence of wild type vs vaccine strain CMV will be performed.

In addition to the CMV PCRs, DNA extracted from the clinical samples will be used to test for a human housekeeping gene, beta-globin, to confirm the integrity of sample collection. Beta-globin testing will stop after 6 months, once samples are demonstrated to be consistently positive. If self-collected samples are not consistently positive or there is concern about declining quality/quantity of DNA isolated, beta-globin testing may be reinstituted.

Viral Neutralization Antibody (NAb) Assay

The purpose of the viral NAb assay is to quantify neutralizing antibody titers to CMV before and after vaccination with CMV vaccine. Functional antibodies will be measured by viral neutralization assay to assess the ability of vaccine-induced immune sera to inhibit viral infection of cells.

This is one of the primary assays used by the Merck Research Laboratories to evaluate the serological response to the vaccine and is currently being validated by an outside vendor to be performed on behalf of Merck Research Laboratories.

A CMV neutralization assay was developed using a report CMV with green-fluorescent protein (GFP) to provide consistent results and flexibility for the screening of large numbers of clinical samples, as needed. In this assay, a virus with a GFP gene inserted into its genome is used to infect ARPE-19cells. GFP levels are measured as an indication of viral infection. Neutralizing antibodies present in test serum prevent the entry of CMV into target cells, prevent expression of the reporter GFP protein and, thus, reduce the signal intensity.

In the viral NAb assay, serum samples are serially diluted and mixed with an epithelial cell tropic HCMV before being added into ARPE-19 cells. Cells are fixed after 48-hour incubation with the serum/virus mixture and then subsequently scanned using an EiSight imager. Data are presented as number of GFP-positive cells per well. The neutralizing activity in the test sera is presented as NT50, which is interpolated dilution corresponding to the 50% of the maximum (median of the no serum control wells) and the minimum (median of the no virus control wells).

Preliminary results using clinical samples from V160-001 showed comparable results to the In-Cell Western neutralization assay used in Phase 1 trial, where the IE-1 protein was used as a read-out by immune-staining on the Li-Cor reader.

IgG ELISA Assay

Viral neutralization assay measures the functional antibodies blocking viral entry and is considered the primary assay for immunogenicity. However, there are certain degrees of

technical challenges to implement the neutralization assay to support a large Phase 3 trial which may enroll over 24,000 subjects. Thus, a viral antigen ELISA measuring surrogate antibody responses is desired.

An ELISA assay measuring the serological responses to purified V160 virus was developed by Merck Research Laboratories and is planned to be validated by an outside vendor to be performed on behalf of Merck Research Laboratories. In this assay, purified V160 virus is coated on microtiter plates. Clinical samples are incubated on the plate and antibodies against HCMV virus will be captured. An HRP-labeled anti-human IgG antibody is used to detect IgG antibodies that are bound to the virus.

Data generated during Phase 2 determine if an ELISA assay is suitable to replace viral neutralization assay in Phase 3.

CMV Cell-Mediated Immunity Assays, CMV IFN-γ ELISPOTs

The purpose of the CMV enzyme-linked immunospot (ELISPOT) assay is to detect interferon gamma (IFN-γ) secreting HCMV-specific cells from peripheral blood mononuclear cells (PBMCs) before and after vaccination with V160. This assay is planned to be performed by an outside vendor on behalf of Merck Research Laboratories to evaluate the cellular immune response to the vaccine. Results for the assay are expressed as the frequency of spot forming cells (SFCs) per million PBMCs.

The IFN-γ ELISPOT assay utilizes 2 high-affinity IFN-γ specific monoclonal antibodies that are directed against different epitopes on the IFN-γ molecule. In the CMV-specific IFN-γ ELISPOT assay, PBMCs are stimulated by the particular CMV antigen in wells of cell culture plates that have been pre-coated with one mouse monoclonal antibody to human IFN-γ. Three different antigens are used to stimulate CMV-specific IFN-γ responses: overlapping 15-mer peptide pools of pp65 and IE1 CMV proteins, and purified CMV virion stocks.

Mitogen and mock-stimulated assay controls will also be performed for each subject. IFN-γ released by the CMV-specific T-cells then binds to the first antibody present in close proximity to the cells that produced it. After ≈18 hours in culture, the cells are washed away, a biotinylated form of the second antibody to IFN-γ is added to the wells of the plate and the plate is incubated overnight at 4ºC. The assay plates are washed, and then alkaline phosphatase-streptavidin is added to each well of the plate. After 2 hours of incubation at room temperature, the plates are washed again and a chromogenic substrate (NBT/BCIP) is added to react with the alkaline phosphatase. As a result, dark blue spots develop against the white background of the plates. The IFN-γ produced by each cell results in the formation of a spot on the culture plate and the number of spots approximates the number of cells that produced IFN-γ in response to CMV antigen. The frequency of SFCs is usually expressed per million input PBMCs. This reflects the T-cell precursor frequency specific to CMV circulating in the blood at a defined time point.

12.6 Appendix 6: Abbreviations and Trademarks

AE	Adverse event/experience
APaT	All participants as treated
CAC	Clinical Adjudication Committee
CI	Confidence intervals
cCMVd	Congenital cytomegalovirus disease
cCMVi	Congenital cytomegalovirus infection
CMVi	Cytomegalovirus infection
CRF	Case report form
CSR	Clinical Study Report
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
ECI	Event of clinical interest
eCRF	Electronic case report form
eDMC	External Data Monitoring Committee
EMA	European Medicines Agency
eVRC	Electronic vaccine report card
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunosorbent spot
EOC	Executive Oversight Committee
ER	Emergency room
ERC	Ethics Committee
FAS	Full Analysis Set
FDAAA	Food and Drug Administration Amendments Act
FK-506	Tacrolimus
GCP	Good Clinical Practice
GFP	Green-fluorescent protein
GMc	Geometric mean count
GMT	Geometric mean titer
β-hCG	Beta-human chorionic gonadotropin
HEENT	Head, eyes, ears, nose, and throat
HIV	Human immunodeficiency virus

HHV-5	Human herpesvirus 5
HRT	Hormone replacement therapy
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICW	In-cell Western
IEC	Independent Ethics Committee
IFN-γ	Interferon gamma
IgG	Immunoglobin G
IM	Intramuscular
IRT	Interactive response technology
IRB	Institutional Review Boards
LLN	Lower limits of normal
MAPA	Merck aluminum phosphate adjuvant
NAb	Neutralizing antibody
PPE	Per-protocol efficacy
PRO	Patient reported outcome
PBMCs	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SAC	Scientific Advisory Committee
SAE	Serious adverse event
SFC	Spot forming cells
SAP	Statistical Analysis Plan
sSAP	Supplemental Statistical Analysis Plan
TC	Telephone contact
US	United States
V160	Merck's candidate CMV vaccine
VE	Vaccine Efficacy
WOCBP	Women of childbearing potential

Supplemental Statistical Analysis Plan (sSAP)

Double-Blind, Randomized, Placebo-Controlled Phase 2b, Multi-center Study to Evaluate the Safety, Tolerability, Efficacy and Immunogenicity of a 2-Dose and a 3-Dose Regimen of V160 (Cytomegalovirus [CMV] Vaccine) in Healthy Seronegative Women, 16 to 35 Years of Age

(Version 2.0)

Prepared by

PPD .

Table of Contents

Listing of Tables

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1. Introduction

This document outlines the statistical analysis strategy and procedures for the V160-002 study. If, after the study has begun, but prior to any unblinding/final database lock, changes made to primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, but prior to unblinding/final database lock, will be documented in this Supplementary Statistical Analysis Plan (sSAP) and referenced in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR.

2. Summary of Changes

2.1 Version 1.0

1. Table 2 in this sSAP, which corresponds to Table 6 in the V160-002 protocol, is expanded to show the decision rules that will be used when the interim analysis (IA) is conducted with more than 15 total CMVi cases in the V160 3-dose and placebo groups combined. Such an eventuality (i.e., over-running of the planned 15 primary efficacy endpoint cases needed for the IA) may happen if more than 15 cases are available in the study database when counting of cases is performed.
   - A counting of total cases of CMVi in the combined V160 3-dose and placebo groups was conducted on 22-November-2019. A total of 6 CMVi cases were obtained.
   - This total number of cases is indicative of an incidence rate that is higher than the anticipated CMVi incidence rate in the placebo group. As such, there is a potential to over-run at the next case counting the 15 CMVi cases needed for conducting the IA.
   - Table 2 in this sSAP is being expanded at this time in order to pre-specify the decision rules, prior to conducting the second counting of total cases of CMVi in the combined V160 3-dose and placebo groups, that will be implemented in an eventuality that the 15 CMVi cases needed for testing the primary efficacy hypothesis at the IA is over-run.

2. Section 3.5.1 enumerates the categories of events, deviations from protocol, or activities that are deemed by the study Clinical Director to potentially interfere with the evaluation of efficacy or immune response to injections of V160, and hence cause a study participant to be excluded from the per-protocol efficacy population.

2.2 Version 2.0

1. Section 3.4.2 no longer has the seroconversion and seropositivity immunogenicity endpoints. These endpoints cannot be defined because both the CMV-specific NAb assay and the IgG ELISA currently do not have identified seropositivity cutoffs that can be used to identify participants who are seropositive based on either of the two assays.

2. Section 3.5.2 provides the definition of the per-protocol immunogenicity (PPI) analysis population.
3. Section 3.6.2 provides the method that will be used in calculating geometric mean titers (GMTs) and the corresponding 95% confidence interval (CI) based on the CMV-specific neutralizing antibody (NAb) assay and IgG enzyme-linked immunosorbent assay (ELISA).
4. Section 3.9.1.1 has a new subsection (Final Testing of the Primary Efficacy Hypothesis) along with a new Table 3 that provides the decision rules that will be applied in the event that a final testing of the primary efficacy hypothesis needs to be conducted at total primary efficacy endpoint case count that is greater than 26 in the combined V160 3-dose and placebo groups (conditional on the IA being conducted at 17 total cases in the combined V160 3-dose and placebo groups).
5. Table 4 in Section 3.9.1.2 has been expanded to cover situations when the secondary efficacy hypothesis is tested at 25 or more (through 35 cases) total cases in the V160 2-dose and placebo groups.

3. Analytical and Methodological Details

3.1 Statistical Analysis Plan Summary

Key elements of the statistical analysis plan are summarized below; the comprehensive plan is provided in Sections 3.2 to 3.12 of this sSAP. For the purposes of analysis and reporting, the overall study ends when all study efficacy, immunogenicity, and safety data are available and ready for database lock execution.

Study Design Overview	Double-Blind, Randomized, Placebo-Controlled Phase 2b, Multi-center Study to Evaluate the Safety, Tolerability, Efficacy and Immunogenicity of a 2-Dose and a 3-Dose Regimen of V160 (Cytomegalovirus [CMV] Vaccine) in Healthy Seronegative Women, 16 to 35 Years of Age
Treatment Assignment	Participants will be randomized in a 1:1:1 allocation to one of the following 3 treatment arms: • V160 3-dose regimen: will receive 3 doses of V160 administered IM according to a Day 1, Month 2, Month 6 schedule; • V160 2-dose regimen: will receive 2 doses of V160 administered IM according to a Day 1 (V160), Month 2 (placebo), Month 6 (V160) schedule; • Placebo regimen: will receive 3 doses of saline solution placebo administered IM according to a Day 1, Month 2, Month 6 schedule. No stratification based on age, sex or other characteristics will be used in this trial.

Analysis Populations	**Efficacy**: Per-Protocol Efficacy (PPE) **Safety**: All Participants as Treated (APaT)
Primary Endpoint(s)	1. **Efficacy**: number of participants with CMVi in the V160 3-dose regimen and placebo arms. 2. **Safety**: number of participants experiencing solicited injection site AEs within Days 1 through 5 after each vaccination visit; solicited systemic AEs and vaccine-related serious AEs within Days 1 through 14 after each vaccination visit.
Key Secondary Endpoints	**Efficacy**: number of participants with CMVi in the V160 2-dose regimen and placebo arms.
Statistical Methods for Key Efficacy Analyses	The primary and secondary efficacy hypotheses will be evaluated by calculating the efficacy of V160 compared to placebo with respect to the CMVi endpoint. The p-value for testing the hypothesis that VE>0% as well as estimation of the 95% CI of VE will be based on the exact binomial method proposed by Chan and Bohidar [1].
Statistical Methods for Key Safety Analyses	There are no *a priori* clinical events identified in this trial as Tier 1 events. Tier 2 events identified in this trial include solicited injection site and systemic AEs; vaccine-related AEs (systemic or serious); any serious AEs (in trial participants or infants born to trial participants); discontinuations due to AEs, AEs by system organ class observed in ≥7 participants in at least one vaccination group and elevated temperatures. Estimates of 95% confidence intervals for between-treatment differences in the percentage of participants with events will be calculated using the Miettinen and Nurminen method [2].
Interim Analyses	One IA will be performed in this trial relating to the testing of the primary efficacy hypothesis. Results will be reviewed by an eDMC. A summary of the IA is provided below. Further details are provided in Section 3.7. **IA**: • Timing: To be conducted upon accumulation of 15 CMVi cases in the combined V160 3-dose regimen and placebo arms, expected at ≈18 months from trial start. • Testing: The primary efficacy hypothesis will be tested. **Final analysis** • Timing: If the result of testing the primary efficacy hypothesis during the IA was inconclusive, a final analysis will be conducted upon accumulation of 24 CMVi cases in the

	combined V160 3-dose regimen and placebo arms, expected at ≈3 years from trial start.
Multiplicity	The primary efficacy hypothesis will be tested once at an IA, and if necessary, a second time at a final analysis. The secondary efficacy hypothesis will be tested in one final analysis, without an IA, only if the primary efficacy hypothesis is successfully demonstrated. The overall one-sided trial Type 1 error associated with testing the primary and secondary efficacy hypotheses is ≤2.5% based on a gatekeeping procedure described in Section 3.8.
Sample Size and Power	The power of the decision rules that will be implemented in this trial for testing of the primary efficacy hypothesis is based on a fixed-event design. • Corresponding to the decision rules shown in Figure 1 in Section 3.7, the trial has >90% power to demonstrate that the efficacy of a V160 3-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥80% at an overall one-sided Type 1 error equal to 2.3% (see Table 2). The trial will randomize 2,100 participants equally allocated into one of the 3 treatment arms as described in the **Treatment Assignment** panel of this section. Under study design assumptions described in Section 3.9.1, the IA of the primary efficacy hypothesis is expected to be conducted at ≈18 months from trial start; and the final analysis if needed is expected to be conducted at ≈3 years from trial start, as mentioned in the **Interim Analyses** panel of this section. The power of the decision rules that will be implemented in this trial for testing of the secondary efficacy hypothesis is also based on a fixed-event design. • Corresponding to the decision rules shown in Figure 1 in Section 3.7, if the secondary efficacy hypothesis is tested based on a total of ≥15 observed CMVi cases in the combined V160 2-dose and placebo regimens, then the trial has power >85% at an overall one-sided Type 1 error ≤2.5% to demonstrate that the efficacy of a V160 2-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥85% (see Table 3).

3.2 Responsibility for Analyses/In-House Blinding

This trial will be conducted as a double-blind trial under in-house blinding procedures.

The statistical analysis of the data obtained from this trial will be the responsibility of the Clinical Biostatistics department of the Sponsor.

The Clinical Biostatistics department will generate the randomized allocation schedules for assignment of study treatment and selection of immunogenicity sub-study participants as described in Section 7.3 of the V160-002 protocol. Randomization will be implemented using IRT.

An external statistician and scientific programmer will be identified and will comprise the unblinded team. This team will be unblinded on a participant-level. They will not be involved in any discussions regarding modifications to the protocol, statistical methods, identification of protocol deviations, or data validation efforts at any time during the trial.

This trial has one planned IA, and its key aspects are described in Section 3.7 of this sSAP. The eDMC will serve as the primary reviewer of the results of the IA. Treatment-level results of the IA will be provided by the external unblinded statistician to the eDMC. The eDMC will make recommendations relating to trial discontinuation or modification to an Executive Oversight Committee (EOC) (see Appendix 1) of the Sponsor. If the eDMC recommends modifications to the design of the protocol or discontinuation of the trial, the EOC may be unblinded to results at the treatment level in order to act on these recommendations. If necessary, limited additional Sponsor personnel may be unblinded to the treatment level results of the IA in order to act on the recommendations of the eDMC. The extent to which individuals are unblinded with respect to results of IA will be documented by the external unblinded statistician. Additional logistical details will be provided in the eDMC Charter.

Upon reaching the milestone when definitive disposition of the primary and secondary efficacy hypotheses has been reached:

- A database lock will be executed.
- All Sponsor personnel who are members of the study team and the V160 program team will be unblinded and a clinical study report will be written.
- If this milestone is reached at or beyond study Year 3, or if the outcome in this milestone is failure of the test of the primary efficacy hypothesis, then the study investigators and participants will be unblinded upon completion of the clinical study report.

Definitive disposition of the primary and secondary hypotheses is reached when one of the following study outcomes occur:

1. Test of the primary efficacy hypothesis fails.
2. Tests of both the primary and secondary efficacy hypotheses succeed.
3. Test of the primary efficacy hypothesis succeeds but the test of the secondary efficacy hypothesis fails.

If at least the primary efficacy hypothesis is successfully demonstrated prior to reaching study Year 3, the study may continue to follow participants up to study Year 3 in order to continue to accumulate more information relating to vaccine efficacy against CMVi and cCMVi. Under this scenario, study investigators and participants will remain blinded during the continuing follow-up for CMVi and cCMVi information. Upon reaching the third year of the study:

- A database lock will be executed.
- An end-of-study clinical report will be written.
- The study investigators and participants will be unblinded upon completion of the end-of-study clinical report.

For the purpose of external reporting of study results in clinical trial registries (eg, EudraCT and Clinicaltrials.gov), the end-of-study milestone corresponds to the last data generated date, which is the date when all study efficacy, immunogenicity, and safety data are available and ready for database lock execution.

3.3 Hypotheses/Estimation

Objectives and hypotheses of the study are stated in Section 4 of the V160-002 protocol.

3.4 Analysis Endpoints

The efficacy, immunogenicity, and safety endpoints that will be evaluated are listed below.

3.4.1 Efficacy Endpoints

The efficacy endpoint that will be used to evaluate VE and test the primary and secondary efficacy hypotheses is the number of participants with primary CMVi, described in Section 4 and Section 5.4.1 of the V160-002 protocol.

CMVi is defined as detection of CMV DNA by PCR in urine or saliva samples.

As stated in Section 4 of the V160-002 protocol, in the evaluation of the primary and secondary efficacy hypotheses, cases of CMVi will be counted starting after the last dose of the assigned vaccination regimen. As stated in Section 3.5.1, the primary and secondary efficacy hypothesis will be evaluated on the PPE population, where eligibility includes being PCR negative post Day 1 through Month 7. Thus, CMVi cases that will be included in the PPE testing of the primary and secondary efficacy hypothesis are those that occurred after Month 7.

3.4.2 Immunogenicity Endpoints

All immunogenicity endpoints in this study are exploratory endpoints and are listed below.

- GMTs based on the NAb and the IgG ELISA assays at Day 1, Months 7, 12, 24, and 36.
- GMc of SFC/10^6 PBMCs based on the ELISPOT assay Day 1, Months 6, 7, 12, and 24.

3.4.3 Safety Endpoints

As specified in Sections 4 and 5.4.1.3 of the V160-002 protocol and Section 3.6.3 of this sSAP, the endpoints that will be used to assess the safety and tolerability of 2-dose and 3-dose regimens of V160 include the following:

- Number of participants experiencing solicited injection site AEs within Days 1 through 5 after each vaccination visit; solicited systemic AEs (including tiredness, muscle pain, headache, and joint pain); and vaccine-related systemic AEs, SAEs, and elevated temperatures within Days 1 through 14 after each vaccination visit
- Incidence of any SAE at any time during the study
- Incidence of vaccine-type viral shedding
- Outcomes of pregnancies.

3.5 Analysis Populations

3.5.1 Efficacy Analysis Populations

Per Protocol Efficacy (PPE)

The PPE population will serve as the primary population for the evaluation of VE and for testing the primary and secondary efficacy hypotheses. To be eligible for inclusion in the PPE population, randomized study participants must satisfy the following criteria:

- CMV seronegative at Day 1 and CMV negative by PCR for non-vaccine strain virus from post Day 1 through Month 7
- Have received all 3 injections/vaccinations within the vaccination visit window specified in the SoA (Section 2)
- At any time from Day 1 through Month 7, did not have events, deviations from protocol, or engaged in activities that are deemed by the study Clinical Director to potentially interfere with the evaluation of efficacy or immune response to injections of V160.

Participants whose CMV status by PCR post Day 1 through Month 7 is not evaluable will be ineligible for inclusion in the PPE population. The categories of events, deviations from protocol, or activities that are deemed by the study Clinical Director to potentially interfere with the evaluation of efficacy or immune response to injections of V160 include the following:

1. Participant was not serologically confirmed as CMV seronegative (based on CMV IgG) during screening;

2. Participant was immunocompromised or had been diagnosed as having a congenital or acquired immunodeficiency, human immunodeficiency virus (HIV) infection, lymphoma, leukemia, systemic lupus erythematosus, rheumatoid arthritis, juvenile rheumatoid arthritis, inflammatory bowel disease, or other autoimmune condition at any time between Day 1 to Month 7;

3. Participant had previously received a non-study CMV vaccine.

4. Participant received systemic corticosteroids (equivalent ≥2 mg/kg total daily dose of prednisone or ≥20 mg/d for persons weighing >10 kg) for ≥14 consecutive days and has not completed treatment at least 30 days prior to any vaccination dose;

5. Participant received systemic corticosteroids exceeding physiologic replacement doses (approximately 5 mg/d prednisone equivalent) within 14 days prior to any vaccination dose;

6. Participant received any anti-viral agent with proven or potential activity against CMV (e.g. letermovir, ganciclovir, valganciclovir, foscarnet, cidofovir, valacyclovir) two weeks prior to any vaccination dose or likely to receive such an agent within two weeks after any vaccination dose.

7. Participant was receiving or has received in the year prior to randomization immunosuppressive therapies including but not limited to rapamycin (also sirolimus), tacrolimus (also FK-506 or Fujimycin), or other therapies used for solid organ/cell transplant, radiation therapy, immunosuppressive/cytotoxic immunotherapy, chemotherapy and other immunosuppressive therapies known to interfere with the immune response.

8. Participant used topical tacrolimus within 2 weeks before or after any vaccination dose;

9. Participant received study clinical material in a sequence that is different from the sequence corresponding to the vaccination group to which the participant was randomized into (i.e., incorrect vaccination sequence or cross-treatment).

10. Participant was administered improperly stored study clinical material that was deemed unacceptable for use.

11. Participant received improperly reconstituted study clinical material that may affect efficacy and/or safety.

Full Analysis Set (FAS)

The FAS will serve as a supportive analysis population for the evaluation of VE. The FAS population consists of all randomized participants who received at least 1 dose of the correct clinical material corresponding to the treatment regimen the participants were randomized into.

3.5.2 Immunogenicity Analysis Populations

Per Protocol Immunogenicity (PPI)

The PPI population will serve as the primary population for the evaluation of immunogenicity. To be eligible for inclusion in the PPI population, randomized study participants must satisfy the following criteria:

- Eligible to be included in the PPE population as defined in Section 3.5.1;
- Have provided serum sample for NAb and IgG ELISA evaluation at 1 month post last dose (Month 7) within the visit window specified in the SoA (i.e., 21 to 49 days after dose 3 of V160 or placebo).

3.5.3 Safety Analysis Population

All Participants as Treated (APaT)

The APaT population will serve as the primary population for evaluation of safety. The APaT population includes all randomized participants who received at least 1 injection of V160 or placebo, have safety follow-up data, and assigns participants to the treatment arm corresponding to the actual clinical material received. For participants who received injections of V160 or placebo corresponding to a sequence that does not correspond to any of the protocol-defined treatment arms (ie, cross-treated participants), a safety profile listing will be created separate from the safety reports that will be provided for the protocol-defined treatment arms.

3.6 Statistical Methods

Statistical testing and inference relating to efficacy are described in Section 3.6.1. Statistical methods relating to safety analyses are described in Section 3.6.3. Efficacy results that will be deemed to be statistically significant after consideration of the Type 1 error control strategy are described in Section 3.8. Nominal p-values may be computed for other efficacy analyses, but should be interpreted with caution due to potential issues of multiplicity, sample size, etc. Unless otherwise stated, all statistical tests will be conducted at the α=0.025 (1-sided) level.

3.6.1 Statistical Methods for Efficacy Analyses

To address the primary and secondary efficacy hypotheses, one-sided tests of the null hypothesis that the vaccine efficacy is 0% versus the alternative that vaccine efficacy is >0% will be conducted separately for each of the primary and secondary hypotheses. Each of the primary and secondary efficacy hypotheses will be tested at one-sided α=0.025 level (ie, 2.5% Type 1 error). Vaccine efficacy is defined as:

$$VE = 100\%*\{1 - (Rv/Rp)\}$$

where Rv and Rp are the incidence rates CMVi in the V160 dose regimen (3-dose for the primary efficacy hypothesis testing; 2-dose for the secondary efficacy hypothesis testing) and placebo groups, respectively. The incidence rate Rv is defined as Rv = Cv/Tv, where Cv = the count of CMVi cases in the vaccine group and Tv = total person-years of follow-up for efficacy in the vaccine group. The incidence rate Rp is defined similarly. The null hypothesis that vaccine is not efficacious (ie, VE=0%) will be tested by constructing a 100*(1–α)% confidence interval (CI) for VE, denoted as (VE_L, VE_U), based on study data at a particular analysis time point (eg, at IA or at final analysis). The statistical criterion for success with respect to the primary efficacy hypothesis (and likewise for the secondary efficacy hypothesis) will be met if VE_L >0%.

The 100*(1–α) % CI for VE, (VE_L, VE_U), is constructed as follows. Under the assumption that R_v and R_p are the means of independent Poisson processes, and given that there is a total of n = Cv + Cp efficacy endpoint cases observed in the combined V160 dose regimen and placebo groups, then the number of primary efficacy cases Cv in the V160 dose regimen group is distributed as Binomial(n,π), where the binomial probability π is defined as π = TvRv/(TvRv+TpRp). The probability π is a person-years-adjusted estimate of the probability

that a particular study participant who became a case of CMVi belongs to the V160 dose regimen group. The lower bound of the 100*(1−α) % exact CI for the probability π is obtained by searching for the proportion π_L such that the probability of observing Cv or more efficacy endpoint cases out of n total efficacy cases is ≤α/2. Similarly, the upper bound of the 100*(1−α)% exact CI for the probability π is obtained by searching for the proportion π_U such that the probability of observing Cv or fewer efficacy endpoint cases out of n total efficacy cases is ≤α/2 [1]. VE_L and V_E are then calculated from π_L and π_U as follows:

$$VE_L = 100\%*\{1- \pi_U (1+\theta)\}/(1 - \pi_U);$$
$$VE_U = 100\%*\{1- \pi_L (1+\theta)\}/(1 - \pi_L);$$

where θ = Tp/Tv is the ratio of the total person-years of follow-up for efficacy in the placebo group over the V160 dose regimen group.

There is only one final analysis of the secondary efficacy endpoint. However, the testing of the secondary efficacy hypothesis may occur at the time of the IA or the final analysis of the primary efficacy hypothesis. The secondary efficacy hypothesis will only be tested if the primary efficacy hypothesis is successfully demonstrated. In addition, the secondary efficacy hypothesis will be tested contingent on accumulation of ≥15 total CMVi cases in the V160 2-dose and placebo regimens (see Figure 1 in Section 3.7, and Section 3.9.2). Thus, the possible timing for testing the secondary hypothesis is as follows.

- If the primary hypothesis test is successful at the IA *and* there are ≥15 total CMVi cases in the V160 2-dose and placebo regimens at the time of IA, then the secondary efficacy hypothesis will be tested immediately after the testing of the primary efficacy hypothesis at the time of the IA.
- If the primary hypothesis test is successful at the IA and there are <15 total CMVi cases in the V160 2-dose and placebo regimens at the time of IA, the secondary efficacy hypothesis will not be tested at the IA. In this case, follow-up will continue until at least 15 total CMVi cases in the V160 2-dose and placebo regimens groups are accrued. Upon accrual of ≥15 total CMVi cases in the V160 2-dose and placebo regimens, the secondary efficacy hypothesis will be tested.
- If the primary hypothesis test is not successful at the IA, the secondary efficacy hypothesis will not be tested at the time of the IA.

3.6.2 Statistical Methods for Immunogenicity Analyses

Within a vaccination group, GMT and the corresponding 95% CI will be derived by taking the anti-logarithm of the mean of the log-transformed antibody titers and its corresponding 95% CI (calculated based on the t-distribution). Antibody titers reported as less than the lower limit of quantitation (LLOQ) of the relevant assay will be replaced by the half of the LLOQ in the log-transformation of antibody titers.

Geometric mean concentration (GMc) of ELISPOT measures and the corresponding 95% CI will be calculated similar to the calculations of GMT and its corresponding 95% CI.

3.6.3 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters including AEs and outcomes of pregnancies.

The analysis of safety results will follow a tiered approach (Table 1). The tiers differ with respect to the analyses that will be performed. Safety parameters or adverse events of special interest that are identified *a priori* constitute "Tier 1" safety endpoints that will be subject to inferential testing for statistical significance with p-values and 95% confidence intervals provided for between-group comparisons. Other safety parameters will be considered Tier 2 or Tier 3. Tier 2 parameters will be assessed via point estimates with 95% confidence intervals provided for V160 dose regimen versus placebo comparisons; only point estimates by treatment group are provided for Tier 3 safety parameters. Safety results will be summarized for each vaccination dose and across all three vaccination doses.

There were no SAEs nor vaccine-type viral shedding (which is an event of interest for V160) observed in the V160 Phase I study. While vaccine-type viral shedding is an event of interest that can occur only in V160-vaccinated subjects, barring false-positive tests in placebo recipients, the 0% incidence of vaccine-type viral shedding observed in the Phase I study provides no informed, data-driven, non-zero incidence level around which a safety hypothesis can be formulated and tested. Therefore, there is no *a priori* identified AE or ECI for which a test of hypothesis will be conducted. Thus, no Tier 1 event is identified in this study. Categories of events considered as Tier 2 and Tier 3 in this study are shown in Table 1. In addition, AEs observed in at least 7 participants (representing 1% of N=700 participants per group) in any vaccination group will be categorized as a Tier 2 event. Incidences of the categories of events identified in Table 1 will be reported as percentages, generally calculated as 100% x (number of participants who experienced the event/total number of participants with safety follow-up), except for outcomes of pregnancies, SAEs throughout the study duration, and vaccine-type viral shedding. The 95% CIs will be provided for vaccine versus placebo percentage point differences (percent in V160 dose regimen group minus percent in placebo) will be calculated using the Miettinen and Nurminen method [2], an unconditional, asymptotic method. No stratification factor will be used in the calculation of 95% CI.

Percentages of outcomes of pregnancies will be generally calculated as 100% x (total number of *specific* pregnancy outcome being reported/total number of *all relevant* pregnancy outcomes). For example, the percent associated with the pregnancy outcome of *live births* will be calculated as 100% x (total number of *live births*/total number of *fetuses/infants with known* outcomes). The numerator and denominator account for pregnancies with multiple fetuses (ie, twins, triplets, etc.) and do not treat such occurrences as a single pregnancy outcome or event. Further details will be provided in the sSAP that will be written for this study.

Vaccine-type viral shedding will be assessed in V160-vaccinated participants. In addition to the analysis strategy shown in Table 1, point and corresponding 95% CI estimates of incidence of vaccine-type viral shedding (number of participants observed with vaccine-type viral shedding/total person-years of follow-up) will be calculated separately for the V160 3-dose, V160 2-dose, and placebo arms. No treatment group comparisons (V160 vs. placebo or V160

3-dose vs. 2-dose arms) of the percent of participants detected with vaccine-type viral shedding will be conducted.

Summaries of SAEs (throughout the study duration) by system organ class will be provided as incidence rates (number of participants [or infants] observed with a specific SAE/total person-years of follow-up) rather than percentages.

Table 1 Analysis Strategy for Safety Parameters

Safety Tier	Safety Endpoint	p-value	95% CI for Comparison of V160 to Placebo	Descriptive Statistics
Tier 2	Solicited injection site AEs[a]		X	X
	Solicited systemic AEs		X	X
	Vaccine-related systemic AEs		X	X
	Any serious AEs (in study participants or infants born to study participants)		X	X[b]
	Vaccine-related serious AEs within Days 1 through 14 after vaccination		X	X
	Discontinuations due to AEs		X	X
	Elevated temperature[c]		X	X
	AEs by SOC observed in ≥7 participants in at least one vaccination group		X	X
Tier 3	AEs by SOC			X
	Outcomes of pregnancies			X
	Vaccine-type viral shedding			X‡

[a] All injection site AEs are considered vaccine-related.
[b] Incidence rates (cases/total person-years follow-up) will be provided instead of percentages.
[c] Defined as ≥38.0ºC [≥100.4°] oral or equivalent
AE = Adverse event; CI = Confidence interval; SOC = System organ class; X = indicated summary statistic will be provided.

3.7 Interim Analyses

3.7.1 Efficacy Interim Analysis

An IA for testing the primary efficacy hypothesis will be conducted upon accrual of a total of 15 CMVi endpoint cases in the combined V160 3-dose and placebo regimens. Possible outcomes of this IA and consequent study pathways are as described below and shown in schematic form in Figure 1.

1. ≥7 of 15 CMVi cases observed in the V160 3-dose arm
   - Study fails to demonstrate the primary efficacy hypothesis at the IA and has no chance of successfully demonstrating the primary efficacy hypothesis in a final analysis when a total of 24 CMVi cases are accrued in the combined V160 3-dose regimen and placebo groups.
   - eDMC may recommend the study to be terminated for futility.
2. (4, 5, or 6) of 15 CMVi cases observed in the V160 3-dose arm.

- Result of testing of the primary efficacy hypothesis is inconclusive.
- Study fails to demonstrate the primary efficacy hypothesis at the IA but still has a chance of demonstrating the primary efficacy hypothesis in a final analysis when a total of 24 CMVi cases are accrued in the combined V160 3-dose regimen and placebo groups.
- Study (which is still blinded) will continue until a total of 24 CMVi cases are accrued in the combined V160 3-dose regimen and placebo groups, at which time, the final testing of the primary efficacy hypothesis will be conducted.

3. ≤3 of 15 CMVi cases observed in the V160 3-dose arm

- Study successfully demonstrates the primary efficacy hypothesis.
- The appropriate timing for the testing the secondary efficacy hypothesis is evaluated (see Section 10.6.1).

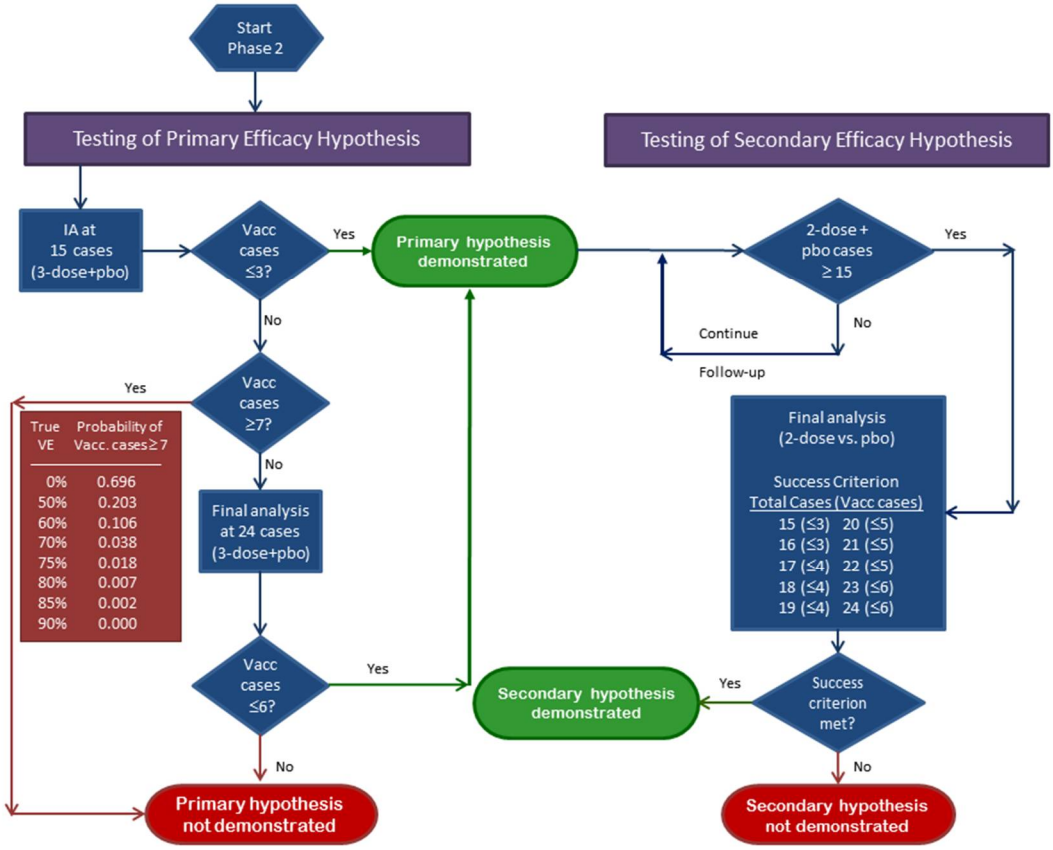

Figure 1 Plan for Testing the Primary and Secondary Efficacy Hypotheses

3.7.2 Safety Interim Analysis

At the time of IA for efficacy, the eDMC will also conduct a review of the cumulative study safety data recorded in the study database upon which the 15 CMVi cases was counted and confirmed to have been accumulated. No hypothesis relating to safety will be tested.

Summaries of safety parameters identified in Section 10.6.3 Statistical Methods for Safety Analyses, will be prepared for review by the eDMC.

3.8 Multiplicity

The overall 1-sided Type 1 error in testing the efficacy hypotheses is controlled to not exceed 2.5% based on gatekeeping procedure.

The primary efficacy hypothesis will be tested at an overall (over the IA and final analysis) 1-sided Type 1 error <2.5%, as discussed in Section 3.9.1. The secondary efficacy hypothesis will be tested at 1-sided Type 1 error ≤2.5% only if the primary efficacy hypothesis is successfully demonstrated, as discussed in Section 3.9.2. The primary efficacy hypothesis is a reasonable gatekeeper to the secondary efficacy hypothesis because if a V160 3-dose regimen with last dose at Month 6 is not efficacious, then a V160 2-dose regimen with last dose also at Month 6 is highly unlikely to be efficacious.

3.9 Sample Size and Power Calculations

3.9.1 Sample Size and Power for Efficacy Analyses

3.9.1.1 Sample Size and Power for Testing the Primary Efficacy Hypothesis

The power corresponding to the decision rule that will be implemented in testing the primary efficacy hypothesis is based on a fixed-event design. For testing the primary efficacy hypothesis, participants will be followed until 15 total CMVi cases in the combined V160 3-dose and placebo regimens have been accumulated, at which point, an IA will be conducted. If the testing of the primary efficacy hypothesis is inconclusive at the IA, follow-up will continue until 24 total CMVi cases have been observed in the combined V160 3-dose and placebo regimens. The decision rules to be implemented that will determine success or failure to demonstrate the primary efficacy hypothesis are as shown in Figure 1 in Section 3.7 and in Table 2. Corresponding to these decision rules, the study has >90% power to demonstrate that the efficacy of a V160 3-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥80% at an overall one-sided Type 1 error equal to 2.3%. The operating characteristics of the decision rules with one IA and a final analysis if necessary are evaluated by a 5 million-replication simulation using SAS v9.4. In the simulation conducted, the underlying probability distribution of accumulation of CMVi cases in the V160 3-dose regimen conditional on the total cases in the combined V160 and placebo regimens is based on an exact method under a large sample Poisson distribution assumption proposed by Chan and Bohidar (1998) [1].

This study will randomize 700 participants into the V160 3-dose regimen and 700 into the placebo regimen. The 15 total CMVi cases in the combined V160 3-dose and placebo regimens are projected to be accumulated in ≈18 to 19 months under the following assumptions: 1) all 1,400 participants are enrolled within 18 months; 2) incidence of CMVi in the placebo group is 2% per year; 2) VE=85%, so that the incidence of CMVi in the V160 arm is 0.3% per year; 3) loss-to-follow-up is ≈5% to 10% per year; and 4) participants ineligible for the PPE analysis is ≈2% to 5% of participants randomized. Under the same assumptions, the total of 24 CMVi cases in the combined V160 3-dose and placebo regimens is projected to be accumulated in ≈3 years.

It is possible that at the time when CMVi cases are counted to determine whether a total of 15 cases have been accrued, the actual CMVi cases accumulated will already be greater than 15. In such an eventuality, the IA will be conducted based on the actual number of cases accrued (eg, 16, or 17, or some other count). Table 2 also shows the operating characteristics of decision rules that will be used in the eventuality that the IA is conducted at a total case count greater than 15.

Table 2 Decision Rule and Corresponding Type 1 Error and Power for Testing the Primary Efficacy Hypothesis of VE >0% Against CMVi (3-Dose Regimen vs. Placebo)

Total Cases Available at IA	Analysis Time Point	Total Cases to Accrue	Stop for Futility (Vacc Cases)	Case Split for Success (Vacc:Pbo)	VE (95% CI)[a]	Cumulative Type I Error[b]	True Vaccine Efficacy					
							70%	75%	80%	85%	90%	95%
							Cumulative Power					
15	IA	15	≥7	≤3 : ≥12	75.0 (7.4, 95.5)	1.8%	53.2%	64.8%	76.8%	87.8%	95.9%	99.5%
	Final†	24	≥7	≤6 : ≥18	66.7 (12.3, 89.2)	2.3%	73.0%	83.7%	92.3%	97.5%	99.6%	100%
16	IA	16	≥8	≤3 : ≥13	76.9 (16.0, 95.8)	1.1%	47.7%	59.9%	72.9%	85.4%	94.9%	99.4%
	Final†	26	≥8	≤7 : ≥19	63.2 (8.5, 86.9)	2.1%	78.0%	87.8%	94.9%	98.7%	99.9%	100%
17	IA	17	≥8	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final†	26	≥8	≤7 : ≥19	63.2 (8.5, 86.9)	1.7%	77.3%	87.4%	94.7%	98.6%	99.8%	100%
18	IA	18	≥8	≤4 : ≥14	71.4 (9.0, 93.2)	1.6%	59.6%	71.6%	83.2%	92.5%	98.1%	99.9%
	Final†	26	≥8	≤7 : ≥19	63.2 (8.5, 86.9)	2.3%	78.8%	88.3%	95.1%	98.7%	99.9%	100%
19	IA	19	≥8	≤4 : ≥15	73.3 (16.3, 93.6)	1.0%	54.5%	67.3%	80.1%	90.9%	97.5%	99.8%
	Final†	26	≥8	≤7 : ≥19	63.2 (8.5, 86.9)	1.9%	77.7%	87.7%	94.8%	98.6%	99.9%	100%
20	IA	20	≥8	≤5 : ≥15	66.7 (3.5, 90.5)	2.1%	69.4%	80.4%	89.8%	96.3%	99.3%	100%
	Final†	27	≥8	≤7 : ≥20	65.0 (13.8, 87.5)	2.4%	78.1%	87.9%	94.9%	98.6%	99.9%	100%
21	IA	21	≥9	≤5 : ≥16	68.8 (10.7, 91.0)	1.3%	64.8%	76.9%	87.6%	95.3%	99.1%	100%
	Final†	28	≥9	≤8 : ≥20	60.0 (5.2, 84.8)	2.3%	83.5%	91.7%	97.0%	99.3%	99.9%	100%
22	IA	22	≥9	≤5 : ≥17	70.6 (16.9, 91.5)	0.9%	60.2%	73.3%	85.3%	94.2%	98.8%	100%
	Final†	29	≥9	≤8 : ≥21	61.9 (10.5, 85.4)	1.6%	80.4%	89.9%	96.2%	99.1%	99.9%	100%

[a] Upon completion of the analyses, a multiplicity-adjusted p-value and confidence interval will be calculated using the group-sequential methodology of Jennison and Turnbull (2000).
[b] Type I error and power shown are cumulative over the IA and final analysis.
CI = Confidence interval; IA = Interim analysis; VE = Vaccine efficacy.

Final Testing of the Primary Efficacy Hypothesis

The eDMC conducted the IA on March 17, 2020 based on a total of 17 primary endpoint cases in the combined V160 3-dose and placebo groups. After the IA was conducted, the eDMC recommended the study to continue unchanged. This eDMC recommendation implies:

1. The study did not meet the futility criterion at the IA.
2. The testing of the efficacy hypotheses (or hypothesis if only the secondary efficacy hypothesis needs to be tested at a future time) is paused.
3. It is unknown to the V160-002 blinded study team whether:
    i. The test of the primary efficacy hypothesis succeeded but there are not enough primary efficacy endpoint cases available in the combined V160 2-dose and placebo groups to test the secondary efficacy hypothesis (see Figure 1); OR
    ii. The test of the primary efficacy hypothesis is inconclusive so that more cases of the primary efficacy endpoint in the combined V160 3-dose and placebo groups need to be accumulated (see Figure 1).

If the pause in efficacy hypotheses testing is due to 3.ii above, because the IA was conducted at 17 total cases of the primary endpoint in the combined V160 3-dose and placebo groups, then the final testing of the primary efficacy hypothesis will be conducted when at least 26 total cases of the primary endpoint have been accrued (see Table 2). In the event of over running the required 26 total cases of the primary endpoint in the combined V160 3-dose and placebo groups when the available efficacy endpoint cases are counted, Table 3 provides the decision rules that will be applied at the final testing of the primary efficacy hypothesis (conditional on the IA being conducted at 17 total cases in the combined V160 3-dose and placebo groups).

Table 3 Decision Rule and Corresponding Type 1 Error and Power for Testing the Primary Efficacy Hypothesis of VE >0% Against CMVi, Conditional on the IA Being Conducted at 17 Total Cases (3-Dose Regimen vs. Placebo)

Cases Available at Final Analysis	Analysis Time Point	Total Cases to Accrue	Stop for Futility (Vacc Cases)	Case Split for Success (Vacc:Pbo)	VE (95% CI)[a]	Cumulative Type I Error[b]	True Vaccine Efficacy					
							70%	75%	80%	85%	90%	95%
							Cumulative Power					
26	IA	17	≥8[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	26	≥8	≤7 : ≥19	63.2 (8.5, 86.9)	1.7%	77.3%	87.4%	94.7%	98.6%	99.8%	100%
27	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	27	≥8	≤7 : ≥20	65.0 (13.8, 87.5)	1.3%	74.2%	85.3%	93.7%	98.3%	99.8%	100%
28	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.5%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	28	≥9	≤8 : ≥20	60.0 (5.2, 84.8)	2.1%	82.7%	91.3%	96.8%	99.3%	99.9%	100%
29	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	29	≥9	≤8 : ≥21	61.9 (10.5, 85.4)	1.6%	80.1%	89.7%	96.1%	99.1%	99.9%	100%
30	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	30	≥10	≤9 : ≥21	57.1 (2.4, 82.7)	2.4%	87.1%	94.1%	98.1%	99.7%	100%	100%
31	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	31	≥10	≤9 : ≥22	59.1 (7.6, 83.4)	1.8%	84.8%	92.9%	97.6%	99.6%	100%	100%
32	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	32	≥10	≤9 : ≥23	60.9 (12.2, 84.1)	1.4%	82.5%	91.5%	97.1%	99.4%	100%	100%
33	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	33	≥11	≤10 : ≥23	56.5 (5.0, 81.5)	2.1%	88.6%	95.1%	98.6%	99.8%	100%	100%
34	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	34	≥11	≤10 : ≥24	58.3 (9.6, 82.2)	1.6%	86.6%	94.1%	98.2%	99.7%	100%	100%
35	IA	17	—[c]	≤3 : ≥14	78.6 (23.2, 96.1)	0.6%	42.4%	54.9%	68.9%	82.8%	93.8%	99.3%
	Final[†]	35	≥12	≤11 : ≥24	54.2 (2.8, 79.7)	2.4%	91.4%	96.7%	99.2%	99.9%	100%	100%

[a] Upon completion of the analyses, a multiplicity-adjusted p-value and confidence interval will be calculated using the group-sequential methodology of Jennison and Turnbull (2000).
[b] Type I error and power shown are cumulative over the IA and final analysis.
[c] The IA was conducted at 17 total cases based on the plan that the final analysis, if needed, would be conducted at 26 total cases. For this plan, the futility criterion at the IA was occurrence of ≥8 cases in the V160 3-dose group. When the IA was conducted on March 17 2020, the futility criterion was not met. If the final analysis needs to be conducted and is conducted at a total of 27 or more cases, the futility criterion at the IA is no longer needed nor relevant since the IA has already been conducted.
CI = Confidence interval; IA = Interim analysis; VE = Vaccine efficacy.

3.9.1.2 Sample Size and Power for Testing the Secondary Efficacy Hypothesis

The power corresponding to the decision rule that will be implemented in testing the secondary efficacy hypothesis is based on a fixed-event design. The secondary efficacy hypothesis will be tested when a total of ≥15 CMVi cases in the combined V160 2-dose and placebo arms have been observed (see Figure 1 in Section 3.7). When the secondary efficacy hypothesis is tested based on a total of ≥15 CMVi cases, the study has >85% power to demonstrate that the efficacy of a V160 2-dose regimen compared to placebo to prevent CMVi is >0% if the underlying vaccine efficacy is ≥85% at an overall one-sided Type 1 error ≤2.5% (see Table 4). The calculations are based on an exact method under a large sample Poisson distribution assumption proposed by Chan and Bohidar (1998) [1] and carried out using the R software.

This study will randomize 700 participants into the V160 2-dose regimen and 700 into the placebo regimen. Under the same assumptions governing the accumulation of CMVi cases in the V160 3-dose and placebo arms, a total of 15 CMVi cases in the combined V160 2-dose and placebo arms are projected to be accumulated in ≈18 to 19 months; and a total of 24 in ≈3 years.

It is possible that at the time when CMVi cases are counted to determine whether a total of 15 cases have been accrued in the combined V160 2-dose and placebo arms, the actual CMVi cases accumulated will already be greater than 15. In such an eventuality, the testing of the secondary efficacy hypothesis will be conducted based on the actual number of cases accrued (eg, 16, or 17, or some other count). Table 4 also shows the operating characteristics of decision rules that will be used in the eventuality that the testing of the secondary efficacy hypothesis is conducted at a total case count greater than 15.

Table 4 Decision Rule and Corresponding Type 1 Error and Power for Testing the Secondary Efficacy Hypothesis of VE >0% Against CMVi (2-Dose Regimen vs. Placebo)

Analysis Time Point	Total Cases Accrued	Case Split for Success (Vacc:Pbo)	VE (95% CI)	Type I Error	True Vaccine Efficacy					
					70%	75%	80%	85%	90%	95%
					Power					
Final	15	≤3 : ≥12	75.0 (7.4, 95.5)	1.8%	53.2%	64.8%	76.8%	87.8%	95.9%	99.5%
	16	≤3 : ≥13	76.9 (16.0, 95.8)	1.1%	47.7%	59.8%	72.9%	85.4%	94.9%	99.4%
	17	≤4 : ≥13	79.2 (0.4, 92.7)	2.5%	64.7%	75.8%	86.0%	93.9%	98.5%	99.9%
	18	≤4 : ≥14	71.4 (9.0, 93.2)	1.5%	59.6%	71.6%	83.2%	92.5%	98.1%	99.9%
	19	≤4 : ≥15	73.3 (16.3, 93.6)	1.0%	54.5%	67.3%	80.1%	90.9%	97.6%	99.8%
	20	≤5 : ≥15	66.7 (3.5, 90.5)	2.1%	69.3%	80.4%	89.8%	96.2%	99.3%	100%
	21	≤5 : ≥16	68.8 (10.7, 91.0)	1.3%	64.8%	76.9%	87.7%	95.3%	99.1%	100%
	22	≤5 : ≥17	70.6 (16.9, 91.5)	0.8%	60.2%	73.3%	85.3%	94.2%	98.8%	100%
	23	≤6 : ≥17	64.7 (6.2, 88.6)	1.7%	73.2%	84.0%	92.5%	97.7%	99.7%	100%
	24	≤6 : ≥18	66.7 (12.3, 89.2)	1.1%	69.1%	81.1%	90.9%	97.1%	99.6%	100%
	25	≤7 : ≥18	61.1 (2.4, 86.3)	2.2%	79.8%	89.1%	95.5%	98.8%	99.9%	100%
	26	≤7 : ≥19	63.2 (8.5, 86.9)	1.4%	76.4%	86.9%	94.5%	98.5%	99.8%	100%
	27	≤7 : ≥20	65.0 (13.8, 87.5)	1.0%	72.8%	84.4%	93.2%	98.1%	99.8%	100%
	28	≤8 : ≥20	60.0 (5.2, 84.8)	1.8%	82.2%	91.0%	96.7%	99.3%	99.9%	100%
	29	≤8 : ≥21	61.9 (10.5, 85.4)	1.2%	79.1%	89.2%	95.9%	99.1%	99.9%	100%
	30	≤9 : ≥21	57.1 (2.4, 82.7)	2.1%	86.7%	93.9%	98.0%	99.6%	100%	100%
	31	≤9 : ≥22	59.1 (7.6, 83.4)	1.5%	84.2%	92.5%	97.5%	99.5%	100%	100%
	32	≤9 : ≥23	60.9 (12.2, 84.1)	1.0%	81.5%	91.0%	96.9%	99.4%	100%	100%
	33	≤10 : ≥23	56.5 (5.0, 81.5)	1.8%	88.1%	94.9%	98.5%	99.8%	100%	100%
	34	≤10 : ≥24	58.3 (9.6, 82.2)	1.2%	85.9%	93.8%	98.1%	99.7%	100%	100%
	35	≤11 : ≥24	54.2 (2.8, 79.7)	2.0%	91.1%	96.6%	99.1%	99.9%	100%	100%

CI = Confidence interval; VE = Vaccine efficacy.

3.9.2 Sample Size and Power for Safety Analyses

Testing of hypotheses relating to safety will not be conducted in this study. Thus, power and Type 1 error considerations relating to safety analyses are not applicable in this study.

The probability of observing a specific AE in specific treatment arms of this study depends on the number of vaccinated participants with safety follow-up and the underlying incidence of that specific AE in the study population. In each treatment arm, assuming that all 700 participants randomized into the study will have safety follow-up, then there is a >99% chance of observing at least one specific AE if the incidence of that AE is at least 1 in every 150 participants; >90% chance if the incidence is at least 1 in every 300 participants. If no vaccine-related SAEs are observed among the 700 participants randomized in a specific V160 treatment arm, this study will provide 97.5% confidence that the underlying percentage of participants with vaccine-related SAE is ≤0.5% (1 in every 200 participants) among V160 vaccinated participants in that V160 arm. This is based on calculation of a 1-sided 97.5% upper confidence limit of a binomial proportion using the exact binomial method of Clopper and Pearson [3] and was done using PASS 2008. For a sample size of 700 per treatment arm, Table 5 provides the critical split of participants with AEs between V160 and placebo arms for which it will be possible to state with 95% confidence that the risk difference (defined as percent in V160 dose regimen group minus percent in placebo) excludes 0%. For a specific AE, given a sample size of 700 per arm, the minimum total number of participants with AEs for which it is possible to state with 95% confidence that the risk difference excludes 0% is 5. As stated in Section 3.6.3, AEs observed in at least 7 participants (representing 1% of N=700 participants per group) in any vaccination group will be categorized as a Tier 2 event. The 95% CIs of the percentage point differences were calculated using the Miettinen and Nurminen method [2].

Table 5 Critical Counts of Participants with Adverse Events in V160 and Placebo Arms Resulting in the Lower Bound of 95% CI Estimate of Risk Difference to be >0%

Observed Count of Participants with Adverse Events			Risk Difference† (V160 – Placebo)	
Total	V160 (N=700)	Placebo (N=700)	Percentage Points‡	95% CI§
5	5	0	0.7%	(0.1%, 1.7%)
6	6	0	0.8%	(0.3%, 1.8%)
7	7	0	1.0%	(0.4%, 2.0%)
8	7	1	0.9%	(0.1%, 1.9%)
9	8	1	1.0%	(0.2%, 2.1%)
10	9	1	1.1%	(0.3%, 2.3%)
11	9	2	1.0%	(0.1%, 2.2%)
12	10	2	1.1%	(0.2%, 2.4%)
13	11	2	1.3%	(0.4%, 2.5%)
14	11	3	1.1%	(0.1%, 2.4%)
15	12	3	1.3%	(0.3%, 2.6%)
16	13	3	1.4%	(0.4%, 2.8%)
17	13	4	1.3%	(0.2%, 2.6%)
18	14	4	1.4%	(0.3%, 2.8%)
19	15	4	1.6%	(0.4%, 3.0%)
	14	5	1.3%	(0.1%, 2.7%)

Observed Count of Participants with Adverse Events			Risk Difference† (V160 – Placebo)	
Total	V160 (N=700)	Placebo (N=700)	Percentage Points‡	95% CI§
20	15	5	1.4%	(0.2%, 2.9%)

† Defined as percent of participants in the V160 group with adverse events minus the corresponding percent in the placebo group.
‡ Calculated as 100%×(V160 – Placebo)÷700.
§ Calculated based on the Miettinen and Nurminen method.
N = Number of participants randomized.
CI = Confidence interval

3.10 Subgroup Analyses and Effect of Baseline Factors

To determine whether VE is consistent across various subgroups associated with specific baseline participant characteristics, the estimate of the VE (with a nominal 95% CI) for the primary efficacy endpoint (comparing 3-dose regimen versus placebo) and for the secondary efficacy endpoint (comparing 2-dose regimen versus placebo) will be estimated and plotted within each subgroup. Subgroup assessments of VE will be conducted for the following baseline participant characteristics:

- Age category (≤21 vs. >21 years)
- Race (white, non-white)
- Region (US, Ex-US)

For each of the primary and secondary efficacy endpoints, a Forest plot will be produced, which provides the estimated point and 95% CI estimates of VE across the subgroups associated with specific baseline participant characteristics listed above. The consistency of VE across subgroups will be assessed descriptively based on these Forest plots.

3.11 Compliance

3.11.1 Adherence to Scheduled Vaccinations

Compliance to scheduled vaccinations in this study is defined as receipt of all 3 vaccinations of either V160 or placebo. To summarize compliance, the numbers of participants who receive each vaccination will be tabulated. For each of vaccination visits 2 and 3, histograms of the time (in days) of administration of the vaccine or placebo relative to the target vaccination visit will be provided.

3.11.2 Adherence to Scheduled Self-Collection of Urine and Saliva Samples

Accurate and reliable ascertainment of the primary and secondary efficacy endpoints (ie, incidence of CMVi) will depend on compliance of study participants to the self-collection of urine and saliva samples based on the schedule indicated in Section 2. Thus, compliance of study participants to the self-collection of urine and saliva samples will be assessed as follows.

On a participant-level (say the *i*-th participant among the N=2100 study participants), percent compliance (denoted by P_i) of a study participant to the self-collection of urine and/or saliva samples will be calculated as:

$$P_i = \frac{n_i}{M_i} x100\%$$

where

n_i = number of visits for which either a urine or saliva sample was received from the study participant;

M_i = total number of study visits throughout the study for which a urine or saliva sample is expected to be received from the study participant. This number will vary based on the time when the study participant enters the study and the time when the study ends.

For each of the V160 3-dose, V160 2-dose, and placebo arms, point and 95% CI estimates of the geometric mean of the participant-level percent compliance P_i will be calculated to summarize the vaccination group-level compliance of study participants to the self-collection of urine and saliva samples.

This assessment of compliance of study participants to the self-collection of urine and saliva samples will be conducted in both the PPE and FAS analysis populations.

3.12 Extent of Exposure

As indicated in Section 5.5 of the V160-002-02 protocol, participants who will receive an injection of V160 at a vaccination visit will receive a 0.5mL dose containing 100 units of V160. Thus:

- Participants randomized to the 3-dose regimen of V160 are expected to be administered a cumulative total of 1.5 mL containing 300 units of V160 over a 6-months duration;
- Participants randomized to the 2-dose regimen of V160 are expected to be administered a cumulative total of 1.0 mL containing 200 units of V160 over a 6-months duration;
- Participants randomized to placebo are not expected to receive V160.

References

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[3] Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 1934;26(4):404-13.