CITY OF HOPE MEDICAL CENTER
1500 E. DUARTE ROAD
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Department of Hematology and Hematopoietic Cell Transplantation

TITLE: A PHASE II RANDOMIZED,PLACEBO-CONTROLLED, MULTICENTER TRIAL TO EVALUATE PROTECTIVE FUNCTION OF CMV-MVA TRIPLEX VACCINE IN RECIPIENTS OF AN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT

CITY OF HOPE PROTOCOL VERSION: IRB #14295 **Protocol Date: 12/15/2022**

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COH Amendment 08	Protocol Dated 04/18/16	Version 08
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COH Amendment 11	Protocol Dated 07/11/16	Version 11
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COH Amendment 14	Protocol Dated 01/19/17	Version 14
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COH Amendment 17	Protocol Dated 07/18/17	Version 17
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DISEASE SITE: Hematopoietic System

SPONSOR/IND NUMBER: City of Hope / IND# 15792

TYPE : Phase II, Therapeutic

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DEPARTMENT OF HEMATOLOGIC ONCOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: A PHASE II RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER TRIAL TO EVALUATE THE PROTECTIVE FUNCTION OF A CMV-MVA TRIPLEX VACCINE IN RECIPIENTS OF AN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT

CITY OF HOPE PROTOCOL NUMBER: #14295 **VERSION:** 19

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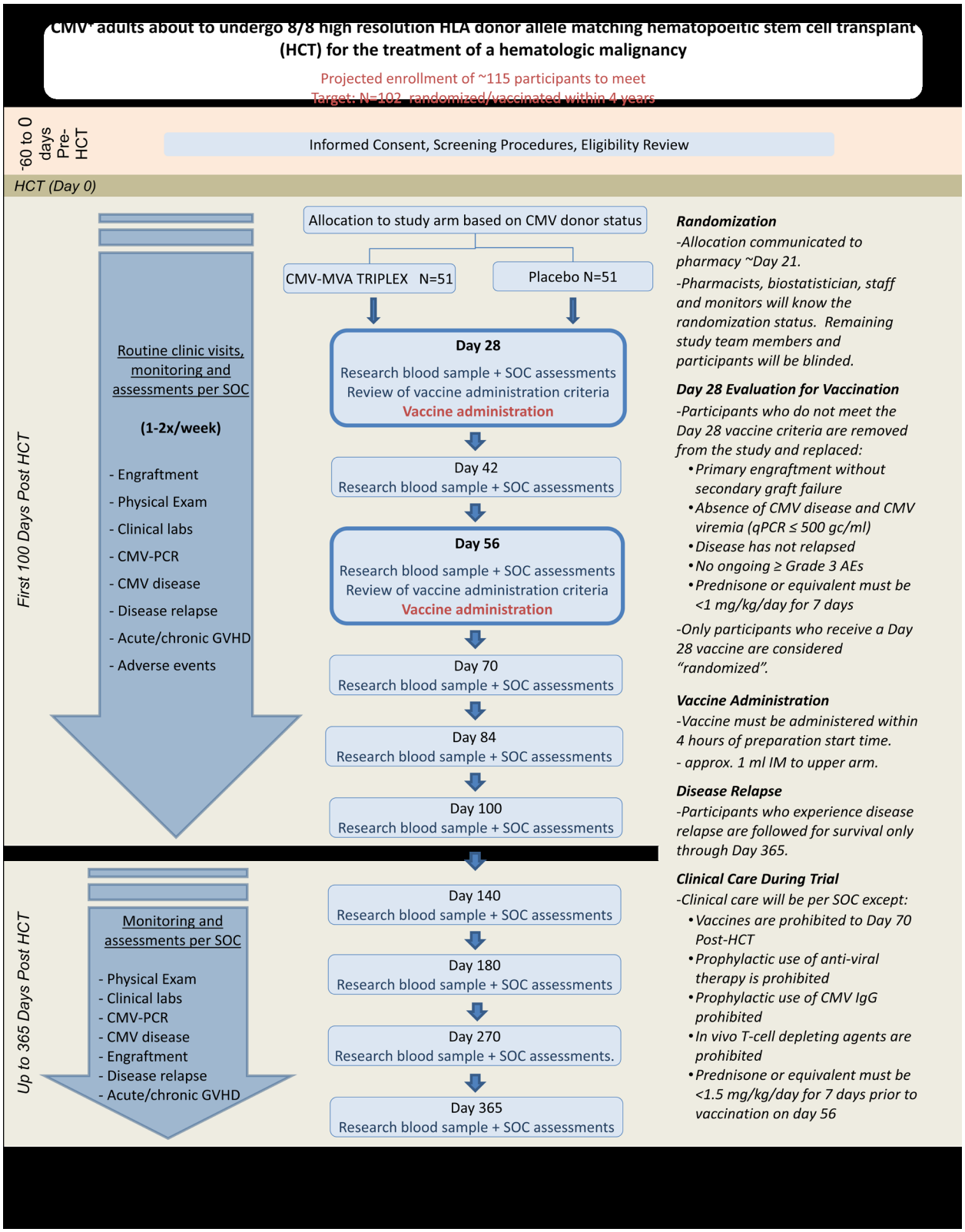
EXPERIMENTAL DESIGN SCHEMA



PROTOCOL SYNOPSIS

Protocol Title:
A Phase II randomized, placebo-controlled trial to evaluate the protective function of a CMV-MVA Triplex vaccine in recipients of an allogeneic hematopoietic stem cell transplant
Brief Protocol Title for the Lay Public (if applicable):
CMV-MVA Triplex, a new vaccine to prevent cytomegalovirus infection after allogeneic stem cell transplantation
Sponsor, IND
COH under IND# 15792
Study Phase:
Phase II, Randomized, Blinded, Interventional
Participating Site:
City of Hope National Medical Center, MD Anderson Cancer Center and Dana-Farber Cancer Institute
Rationale for this Study:
Cytomegalovirus (CMV) is associated with a number of clinical syndromes. [1]. Since the inception of allogeneic hematopoietic cell transplant (HCT) as a cure for hematologic malignancies, one of the main infectious complications during the first 100 days post-transplant is viremia caused by CMV[2-7]. HCT patients are vulnerable to herpes-virus infections, including CMV, as a result of immunosuppression associated with treatment strategies aimed at preventing graft rejection or graft versus host disease (GVHD) [8-10]. While anti-viral agents that limit viral replication can initially control CMV viremia, they are associated with significant toxicity and fail to protect against late-onset CMV disease, including reactivation and failure to reconstitute CMV-specific immunity. Substituting toxic antivirals with a vaccine that harnesses the abundant native immune response to CMV may improve outcomes for HCT recipients[11]. CMV-MVA Triplex is a multiple-antigen recombinant Modified Vaccinia Ankara (MVA) with genes encoding 3 immunodominant CMV proteins: UL83 (pp65), UL123 (IE1), and UL122 (IE2). MVA is attractive as a therapeutic agent due to its safety record as a smallpox vaccine, including in the young and elderly [12]. Recently published data from a randomized, placebo-controlled, double-blind study has shown that MVA was safe, well tolerated and immunogenic when used as a vaccine in HCT recipients[13]. Additionally, previous results strongly support its use as a vector for delivering infectious disease antigens, since MVA vaccination safely induced robust cellular immune responses in HCT recipients [13]. Investigators at COH and collaborating institutions are evaluating CMVPepVax, a novel peptide vaccine developed at City of Hope. A limitation of this peptide-based vaccine is its exclusive use in the HLA A*0201 population that only comprises around 30-40% of patients at risk for CMV post HCT. In contrast, CMV-MVA Triplex vaccine activity is not limited to an HLA restricted HCT recipient population, potentially providing CMV protection to a broader population. Furthermore, MVA

vaccines such as the CMV-MVA Triplex vaccine have a track record for stimulating potent immunogenicity, in most cases far superior than DNA vaccines[17].

These facts motivated the development of an MVA vaccine candidate incorporating immunogenic targets of human CMV. No current vaccine strategy against CMV developed for HCT recipients uses a recombinant vector incorporating multiple cellular response antigens. The CMV-MVA Triplex vaccine is manufactured at City of Hope (COH) Center for Biomedicine and Genetics (CBG), and has been tested in a phase I dose escalation study in healthy volunteers. The vaccine was administered twice in a 28-day period and shown marked safety in healthy adults vaccinated with up to $5x10^8$ pfu/mL. Additionally, CMV-MVA Triplex induced robust expansion of CMV-pp65, IE1 and IE2 CD8 and CD4 T cells in the healthy volunteers. There have been no serious adverse events (SAE) or dose limiting toxicity (DLT).

As safety and preliminary immunogenicity have now been established in healthy adults, there is a strong rationale to perform a Phase II study to assess safety and efficacy of CMV-MVA Triplex in protecting against CMV reactivation and disease in the target HCT patient population at risk of life-threatening complications. Funding for conducting this Phase II trial is from NCI.

Objectives:

Primary Objectives:

- *Safety:* To evaluate the safety and tolerability of CMV-MVA Triplex in vaccinated HCT recipients by assessing the following: non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and grade 3-4 AEs (CTCAE 4.0) probably or definitely related to the vaccination within 2 weeks from each vaccination.
- *CMV events:* To determine if CMV-MVA Triplex reduces the frequency of CMV events defined as reactivation or CMV disease in allogeneic HCT-R^+. A CMV event encompasses - CMV "reactivation" (DNAemia ≥500 gc/mL by qPCR, low levels viremia treated by anti-virals; or detection of CMV by tissue histology (end-organ disease).

Secondary Objectives:

- *Viremia duration and treatment*: To characterize CMV reactivation and CMV disease in recipients of CMV-MVA Triplex compared to placebo by assessing time to viremia (defined as number of days from transplantation to the date of >500 CMV gc/mL), duration of viremia, recurrence of viremia, incidence of late CMV viremia/disease (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia of ≥1500 CMV gc/mL), cumulative number of CMV specific antiviral treatment days.
- *Transplant-related outcomes*: To evaluate the impact of CMV-MVA Triplex on transplant related outcomes by assessing the incidence of acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, infections.
- *Cellular Immunity*: To determine 1) if CMV-MVA Triplex increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated allogeneic HCT-R^+, 2) to determine whether vaccination induces adaptive NK cell population changes, and increase in the highly cytotoxic memory NKG2C$^+$ NK cells, and 3) to explore GVHD biomarkers and compare between the vaccine and placebo groups.

Study Design:

Overall design: This is a multisite (COH, Dana-Farber, MDA), randomized, blinded, placebo-controlled, parallel-group Phase II trial to evaluate: a) safety and reduction in the frequency of CMV reactivation and disease (CMV events) and b) increase in CMV cellular immunity, in allogeneic HCT-R^+ vaccinated with CMV-MVA Triplex relative to those injected with placebo. This trial is designed to 1:1 randomly allocate 102 allogeneic HCT-R^+ at risk for CMV complications to CMV-MVA Triplex arm or placebo arm.

Enrollment and randomization:

Eligible HCT-R+ will be consented and enrolled pre-HCT. A computer-generated randomization, stratified by donor CMV serostatus and center will assign registered participants to the CMV-MVA Triplex or placebo arm; the treatment assignment will be generated and provided to pharmacists, who are unmasked to treatment-group allocation, in advance of planned vaccination. Enrolled participants will be followed for the course of transplant and be assessed for vaccine administration criteria for 'Day-28 post-HCT' vaccination. Participants who meet vaccine administration criteria will receive the vaccine; participants who do not meet criteria will discontinue study participation and be replaced. Only participants who receive a vaccine (CMV-MVA Triplex or placebo) on day 28 will be considered "randomized". Information regarding participants who do not receive vaccine will be entered into the computer-generated randomization program to inform the randomization algorithm for subsequent treatment assignments.

Administration of CMV-MVA Triplex /placebo: CMV-MVA Triplex vaccine or placebo is administered intramuscularly (IM) on days 28 and 56 post-HCT.

Duration of participation: Study participation will be completed on Day 365 Post-HCT for all participants who receive a vaccine injection. Participants who do not receive a vaccine injection will discontinue any further follow-up once the determination is made that the vaccine will not be administered.

Stopping rules: Formal stopping rules will be implemented, two major safety endpoints; non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and Serious AEs (SAE, grade 3-4) related to the vaccination within 2 weeks from each vaccination will be monitored, but based on assessment of individual cases. This study will have an external independent DMC for review of protocol events and progress, with reporting of recommendations to COH IRB.

Endpoints:

Primary endpoints:

CMV events (reactivation [≥500 CMV genome copies (gc)/mL], use of CMV-directed antivirals, or CMV disease) prior to day 100 post-HCT. Timing and recurrence of events are included in the primary analysis.

Key safety endpoints: non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) aGVHD, and grade 3-4 AEs (CTCAE 4.0) probably or definitely related to the vaccination within 2 weeks from each vaccination.

Secondary endpoints:

Viremia treatment and duration: Duration of viremia, incidence of late CMV viremia (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia of ≥1500 CMV gc/mL), cumulative number of CMV specific antiviral treatment days.

Transplant related events: time to engraftment, incidence of aGVHD, chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, infections.

Immunological function: levels and kinetics of CMV-specific T cell immunity, combined with immunophenotyping[19-21], and functional studies[22, 23]. NK phenotype and function (cytotoxicity and cytokine production).

Sample Size:

The target of this Phase II multicenter study is to randomize 102 allogeneic HCT-R^+ to either the CMV-MVA Triplex vaccine arm (N=51), or to the placebo arm (N=51). With the expected drop out rate of 10-15% prior to randomization, the total accrual is expected to be 113-120.

Estimated Duration of the Study

Over the past five years, COH, and Dana-Farber combined have performed >300 adult allogeneic HCT procedures annually. We anticipate about 200 to be eligible annually being CMV seropositive. It is estimated that accrual will be completed in <2 years from the start date of the trial, and that trial we anticipate 1 year of follow up and data analysis.

Summary of Subject Eligibility Criteria:

Pre-HCT Inclusion Criteria

- Age 18 to 75 years.
- Planned HCT for the treatment of hematologic malignancy (some exceptions including multiple myeloma
- Planned related or unrelated HCT, with 8/8 (A,B,C,DRB1) high/intermediate resolution HLA donor allele matching and with minimal to no T cell depletion of graft
- CMV seropositive (recipient)
- Seronegative for HIV, HCV and active HBV

Pre-HCT Exclusion Criteria

- Patients undergoing a second allo HCT are not eligible (patients who have undergone a previous autologous HCT are eligible)
- Prior investigational CMV vaccine, Experimental anti-CMV chemotherapy in the last 6 months
- No planned use of the following after HCT: Live attenuated vaccines, medically indicated subunit or killed vaccines, alemtuzumab or any equivalent in vivo T cell depleting agent, medications with known activity against CMV, CMV immunoglobulin
- Aplastic anemia
- Patients with active autoimmune conditions requiring systemic immunosuppressive therapy within the previous 5 years are not eligible
- Pregnant women and women who are lactating.

Post-HCT Day 28 Vaccine Administration Criteria*

- No ≥ Grade 3 GVHD within 7 days from the day of vaccine
- Disease has not relapsed since HCT
- Successful primary engraftment without secondary graft failure
- No ongoing post-HCT ≥ Grade 3 non-hem AE's, with the exception of grade 3 glucose intolerance, cholesterol, triglyceride, and hyperglycemia
- No for CMV viraemia: CMV qPCR ≤ 500 gc/mL from samples collected and resulted within the past 7 days
- Negative for CMV end organ disease (biopsy proven CMV disease) post-HCT
- All prednisone doses within the past 7 days were ≤ 1 mg/kg/day (or prednisone equivalent)
- Not received any prohibited medications (see Section 5.6)

*Participants who do not meet vaccine administration criteria will be replaced.

Investigational Product Dosage and Administration:

Participants will be randomized to receive either the CMV-MVA Triplex vaccine or the placebo. The pharmacy will know the randomization status of participants, while the clinical study team and participants will remain blinded to the randomization status.

Approximately 5 x 10^8 pfu of Triplex Vaccine (or an equivalent volume of placebo) will be administered IM in the upper arm on Days 28 and 56 post-HCT. There are no dose modifications, although participants must meet vaccine administration criteria in order to receive the vaccine.

CMV-MVA Triplex vaccine is constituted of 5.1 or 9.1 x10^8 pfu/mL in PBS containing 7.5% lactose.

Placebo comprises an isotonic solution of the PBS containing 7.5% lactose.

Clinical Observations and Tests to be Performed:

Clinical observations/clinical tests: medical history, physical exams, performance status, routine laboratory tests (CBC, chemistry panel), CMV qPCR, GVHD assessment, AE assessment, diagnostics for disease relapse assessment (bone marrow and imaging studies), diagnostics for CMV disease assessment, according to institutional standard of care. All of the study endpoints listed above will be monitored and recorded. Clinical CMV disease and use of anti-viral drugs will be prospectively monitored and recorded.

Immunologic studies: Immunologic studies will include monitoring levels, function and kinetics of CMV-specific T cell immunity, combined with immunophenotyping studies [19, 21]. The phenotypic ratios of CMV-specific T cells will be related to improvement in control of CMV viremia. Characterization of highly cytotoxic memory NKG2C$^+$ NK cells[24], linked to CMV reactivation and critical for CMV adaptive immune response will be performed[25-27].

Statistical Considerations:

A 40% rate of viremia (CMV reactivation) is expected among unvaccinated, eligible HCT recipients, with some possibility of a rate as low as 30%. Based on the aim to detect either a vaccine-effect resulting in a drop from 40% to 15% or a drop from 30% to 10%, we plan to enroll and inject 51 subjects in each group. The sample will provide at least 90% power under either scenario, at a one-sided 0.10 level of significance (appropriate to this phase II trial). The sample will allow at least 83% power to achieve a 0.05 level of significance. Allowing for 10% of subjects becoming ineligible before vaccination, we expect to enroll approximately 114 subjects in 2 years, to vaccinate 102, and observe

between 20 and 28 reactivation events.

Analysis Plan: The primary aim of comparing reactivation event rates will use the time-to-event methods incorporating repeated events, as we have previously reported [28]. Computing will be done with the coxph function of the R survival package (Therneau) with implements the counting process formulation of Andersen and Gill. Formal stopping rules for safety are embedded in the design of this study. Clinical data will be monitored by the PMT and external DMC, and investigation of CMV-MVA Triplex will be suspended for safety review if there is evidence of serious treatment-related AEs.

Specifically:
(1) 100 days NRM will be monitored as the 12^{th}, 24^{th} and 36^{th} subject on the vaccine arm reaches the 100 day evaluation point. Operationally, the CRA will notify the monitoring statistician as cohorts of 24 patients (approximately 12 vaccinated) near the 100 day mark. If NRM frequencies exceed 4, 6, or 8, at the designated 100 day evaluation point, then the trial will be suspended for safety review by the COH, MDA, Dana-Farber and External DMCs. These numbers were selected to limit the overall false-alarm probability for this endpoint to less than 0.02 when there is no additional risk due to immunization.

(2) Severe acute GVHD (aGVHD, grade 3-4) will be monitored as every 12^{th} subject on the vaccine arm reaches the 100 day evaluation point. aGVHD will be scored using Keystone consensus criteria[29]. The trial will be interrupted at each respective center for safety review by the COH, MDA, Dana-Farber and External DSMCs if 6 or more of the first 12 recipients, or 9 of 24, or 11 of 36, experience Grade 3-4 aGVHD. This would be a significant elevation from the COH/MDA/Dana-Farber historical benchmark of 15% of allogeneic HCT recipients with matched sibling donors [30]. These rules are determined to limit the overall aGVHD false alarm probability to 0.02 if vaccination does not increase risk [30].

(3) Serious AEs (SAE, grade 3-4) related to the vaccination within 2 weeks from each vaccination will be discussed individually by the PMT and will be reported to the DSMC.

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ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse Event
aGVHD	Acute Graft Versus Host Disease
BDP	Biopharmaceutical Development Program NCI-Frederick, MD
CBC	Complete Blood Count
cGVHD	Chronic Graft Versus Host Disease
cGMP	Current Good Manufacturing Practice
CLIA	Clinical Laboratory Improvement Amendments
CMV	Cytomegalovirus
CMV-MVA Triplex	Cytomegalovirus-Modified Vaccinia Ankara encoding 3 immunodominant CMV proteins
COH	City of Hope
CRA	Clinical Research Associate/Coordinator
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTL	Cytotoxic T lymphocytes
DET	Department of Experimental Therapeutics
DFCI	Dana-Farber Cancer Institute
DSMC	Data Safety Monitoring Committee
ECG	Electrocardiogram
FDA	Food and Drug Administration
FOS	Foscarnet
GCP	Good Clinical Practice
GCV	Ganciclovir
GVHD	Graft versus host disease
LCL	Human B-lymphoblastoid cell lines
HBV	Hepatitis B virus
HCT	Hematopoietic Stem Cell Transplant
HCT-R+	CMV positive HCT recipients
HCV	Hepatitis C virus
HHV6	Human herpes virus 6

HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HSV	Herpes simplex virus
HVTN/DAIDS	HIV Vaccine Trials Network/Division of AIDS
ICS	Intra cellular staining
ID50	Median Infective Dose
IDS	Investigational Drug Service
IFN	Interferon
ICF	Informed Consent Form
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
IVS	*In vitro* stimulation
MDA	MD Anderson
MRD	Matched Related Donor
MUD	Matched Unrelated Donor
MVA	Modified Vaccinia Ankara
NCI	National Cancer Institute
NRM	Non-relapse mortality
OIDRA	Office of IND Development and Regulatory Affairs
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PD-1	Program death receptor-1
pfu	Plaque forming unit
PI	Principal Investigator
R^+	CMV positive HCT recipients
PMT	Protocol Monitoring Team
SAE	Serious Adverse Event
SAIC	Science Applications International Corporation
SOC	Standard Of Care
VAL	Valganciclovir

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

Primary Objectives:

- To evaluate the safety and tolerability of CMV-MVA Triplex in vaccinated HCT recipients by assessing the following: non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and grade 3-4 AEs (CTCAE 4.0) probably or definitely related to the vaccination within 2 weeks from each vaccination.
- To determine if CMV-MVA Triplex reduces the frequency of CMV events defined as reactivation or CMV disease in allogeneic HCT-R^+. A CMV event encompasses any detection of CMV by either qPCR (termed "reactivation": DNAemia ≥500 gc/mL or by tissue histology (end-organ disease).

Secondary Objectives:

1) To characterize CMV reactivation and CMV disease in recipients of CMV-MVA Triplex compared to placebo by assessing time-to viremia (defined as number of days from transplantation to the date of >500 CMV gc/mL), duration of viremia, recurrence of viremia, incidence of late CMV viremia/disease (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia of ≥1500 CMV gc/mL), cumulative number of CMV specific antiviral treatment days.
2) To evaluate the impact of CMV-MVA Triplex on transplant related outcomes by assessing the incidence of acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, infections.
3) To determine 1) if CMV-MVA Triplex increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated HLA A*0201, CMV seropositive HCT-recipients, 2) to determine whether vaccination induces adaptive NK cell population changes, and increase in the highly cytotoxic memory $NKG2C^+$ NK cells, and 3) to explore GVHD biomarkers and compare between the vaccine and placebo groups.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Human CMV is a double-stranded DNA β-herpes virus which is highly prevalent globally, but rarely elicits disease in healthy immunocompetent hosts. The human immune system is unable to clear CMV infection and latency, but mounts a robust response targeting multiple immune-evasion genes. CMV is among the largest and most complex of known viruses, with a genome encoding around 165 genes [31]. The magnitude of the CMV-specific cellular immune response is the most striking aspect of the dynamic, life-long interaction between the host and CMV. CMV-specific T cells are essential to control CMV viral replication and prevent disease, though do not eliminate the virus or preclude transmission.

Significant suppression of host antiviral immunity can alter the life-or-death immune surveillance homeostasis, allowing CMV reactivation to become detectable or primary infection to cause clinical symptoms. Uncontrolled viral replication and dissemination results in the development of life-threatening end-organ damage (CMV disease)[32-34]. CMV infection is the cause of major complications and significant morbidity in the recovery of immune-compromised recipients both at early and late times post-HCT [4, 6, 7]. HCT patients are vulnerable to herpes virus infections, including CMV, as a result of immunosuppression associated with treatment aimed at preventing rejection or GVHD[35-37].

Pharmacologic agents used to limit virus replication, such as, GCV or its oral form VAL, and foscarnet (FOS) are the methods of choice for prophylaxis CMV infection [4, 38]. Despite this, CMV remains an important cause of mortality after HCT diminishing the full curative potential of this successful cancer therapy [20, 39]. Furthermore, anti-viral chemotherapy has major side effects, including nephrotoxicity, neutropenia, and delayed immune reconstitution which exposes HCT recipients to other opportunistic viral, bacterial and fungal infections[7, 40]. For example, the use of GCV/VAL is associated with a higher proportion of recipients becoming neutropenic and increased numbers of concomitant fatal infections [7, 40]. As GCV/VAL therapy has become ubiquitous in practice, delayed onset of CMV-pneumonia (interstitial pneumonitis, IP) is more frequent which suggests that GCV/VAL impairs immunologic reconstitution [41, 42]. When antivirals are stopped or when virus resistance occurs, the same disease symptoms appear; only frame shifted to ~180 days post-HCT [6, 43-45]. Thus new strategies to control CMV are required. Antivirals have limitations, and their use does not address the risks of late-onset CMV disease including early CMV reactivation and failure to reconstitute CMV-specific immunity[39]. Substituting toxic antivirals with a vaccine that harnesses the abundant native immune response to CMV may improve outcomes for HCT recipients. In particular, a CMV vaccine that confers protective immunity early post-transplant, until normal immunocompetence is re-established in the HCT recipient (6 months or earlier post-HCT) may reduce CMV morbidity and the use of antivirals[11].

MVA: The attenuated poxvirus MVA, engineered with recombinant genes, is being evaluated as a clinical vaccine for infectious disease and cancer. The attractiveness of MVA for clinical use stems from its previous safety record as a smallpox vaccine in youngsters and in the elderly[12]. Recent clinical trials have affirmed its safety and efficacy in protecting against malaria challenge and generating immune responses in humans exposed to the TB bacterium [46, 47]. Lack of viral assembly and avirulence in mammals, together with studies showing its safety in heavily immunosuppressed macaques, rodents, and in HIV-AIDS patients supports its use in HCT recipients [48-50]. Recently published data from a randomized, placebo-controlled, double-blind study [Clinical Trials Registration: NCT00565929] has shown that MVA was safe, well tolerated and immunogenic when used as a vaccine in HCT recipients [13]. Additionally, they strongly support its use as a vector for delivering infectious disease antigens, since MVA vaccination safely induced robust cellular immune responses in HCT recipients [13]. Unlike other attenuated poxviruses, the block in viral assembly does not interfere with production of large quantities of recombinant proteins in otherwise non-permissive hosts [51]. Multiple sites of foreign gene integration in MVA allow the virus to be modifed to expres multiple full-length antigens. These

facts motivated the development of vaccine candidates incorporating immunogenic targets of human CMV. No current vaccine strategy against CMV that uses a recombinant vector incorporating multiple cellular response antigens is being developed for HCT recipients. Since human CMV infection is severely host-restricted, it precludes further evaluation of vaccine candidates to protect against clinical disease in animal models. Human testing of vaccines against CMV remains the only valid option to address their potential protective function.

Cellular immune response to CMV in healthy adults: After primary infection, CMV persists under control of cell-mediated immune (CMI) surveillance [52-55]. Using whole genome overlapping peptide libraries, the targets of the healthy human T cell response to CMV were identified [31, 56]. These studies show that the main target for HLA Class 1-restricted T cells is the tegument protein UL83, referred to as pp65 [57-59]. However, immediate early proteins UL123 (IE1) and UL122 (IE2) also are prominent targets for CD8 T cell responses in healthy individuals and patients [31, 60-63]. These 3 targets have a profile that is consistent with protective immunity documented in HCT and SOT recipients [20, 64-66]. Phase 1 and 2 studies showed that pp65- ALVAC™ stimulated primary immunity among CMV-negatives to levels approaching naturally CMV-positive individuals [67]. Similarly, AlphaVax™ confirmed that property for pp65 and IE1 [68], and TransVax™ DNA vaccine to a lesser extent [69]. These results provide a rationale for our choice of CMV pp65, IE1 and IE2 antigens to be incorporated into the CMV-MVA vaccine.

CMV immune response in HCT Recipients: CMV interferes with the proper function of the immune system [18, 69-75], thus altering the host immune response to CMV. Subunit Ag vaccination strategies are the best option to overcome CMV-mediated immunosuppression by limiting viremia, an independent predictor of disease [76]. Several reports have associated protection from CMV disease in HCT recipients with CMV-specific $CD8^+$ T cell levels between 7-10/µL [18, 71, 77]. IE1-specific T cells were found to protect against CMV disease in SOT patients demonstrating the need for both types of T cells for efficient protection [65, 66]. COH studies demonstrated that the $CD8^+$ T cell expansions can occur prior to d40 post-HCT, and in some cases, these T cells have been traced as clonal expansions derived from the donor [72]. Early clinical studies show that $CD8^+$ cytotoxic T lymphocyte (CTL) development is necessary to protect from CMV-IP in HCT recipients [3, 11, 78]. In patients who have measurable CMV viremia, expansion of $CD8^+$ T cells occurs, especially after viral reactivation [71, 73]. In addition, CMV disease occurred only in patients with low level CMV-$tetramer^+$ $CD8^+$ T cells [20, 70, 74]. Immunologic monitoring showed the benefit of CMV-positive donors even in the case of CMV-negative HCT recipients for long term CMV immune reconstitution [18, 75]. Cellular immunity in HCT recipients is augmented during CMV reactivation as shown by increased levels of functional CMV-specific $CD8^+$ T cells [20, 70, 71]. In the largest study of its kind, we evaluated impact of T cell memory on the pace of CMV-specific immune reconstitution. We confirmed results from multiple clinical trials showing that a CMV-positive donor enhances CMV immunity in the recipient and decreases severity and duration of CMV infection [28, 76, 79-81].

Vaccine Strategies against CMV for HCT recipients: The purpose of using live viral vaccination is to induce both helper and cytotoxic immunity, which may lead to a durable memory response[82, 83]. Plotkin and co-workers established an attenuated strain of CMV, the Towne strain, as a proposed therapeutic vaccine in the 1970's, however concerns about using live CMV have minimized its applicability[78, 84]. More problematic for using attenuated CMV as a vaccine is the frequency of acquisition of a new CMV strain under pre-existing strain-specific natural immunity [85]. Alternative live viral approaches for CMV vaccines have focused on canarypox (ALVAC) expressing gB (UL55) which did not elicit significant antibody in CMV-negatives [86, 87], or ALVAC-UL83 (PP65) which stimulated robust cellular immunity in CMV-negatives equivalent to levels in natural CMV-positives [67]. Further studies with ALVAC-UL55 and purified soluble UL55 protein only revealed minimal efficacy, insufficient for licensure [88, 89]. Another approach using AlphaVax™ expressing UL83, UL123 and UL55 [68] showed promise when used in healthy adults. However, it is unlikely that AlphaVax™ can be accepted in HCT, since it is based on a recombinant VEE, a live virus that could propagate in humans [68]. TransVax™ DNA

vaccine vector expressing either UL55 or UL83 have been evaluated in animal models with good results [90-92]. When clinically evaluated, TransVax™ vaccine, which requires multiple injections showed weak immune response in healthy adults, and failed to meet its first endpoint of reduced GCV usage in HCT recipients[93, 94]. CMVPepVax, derived from the CMV-UL83 antigen showed remarkable safety and elicited vaccine driven immune responses when tested in healthy adults (IRB protocol #03121; J. Zaia, P.I.)[15]. CMVPepVax is currently being evaluated in HCT recipients (IRB protocol #12022). In the HCT setting, the study indicated safety of injecting CMVPepVax in HCT recipients on day 28 and day 56 post-HCT, no increase in acute GVHD and reduced CMV reactivation associated with vaccine-stimulated immunity [16]. While the CMVPepVax immunologic activity has shown promise, a limitation of the HLA A*0201 restricted CMVPepVax is that is solely active in the HLA A*0201 population, that only makes up ~30-40% of the at-risk HCT population. The CMV-MVA Triplex vaccine being investigated in this protocol has a superior track record for stimulating potent immunogenicity than does DNA vaccines, and since it expresses whole CMV proteins it has broader recognition and greater applicability for HCT recipients than CMVPepVax, which has a narrow applicability. Thus, the CMV vaccine field has made progress since the 1970's, but a clear-cut strategy in which multi- antigen cellular immunity is stimulated from a single and safe vaccine vector has been elusive, and it is for these reasons that the development and clinical testing of CMV-MVA Triplex vaccine is important.

MVA as a delivery vehicle for vaccines against infectious diseases: The development of MVA as a recombinant vaccine stemmed from its benign safety profile as a smallpox vaccine in Europe in the 1970's [12, 95, 96]. Development into a vaccine vehicle was only initiated in the early 1990's, when it became clear that non-attenuated poxviruses such as the Western Reserve (WR) strain could not be safely administered to immuno-compromised persons [97, 98]. Although MVA is able to efficiently replicate DNA in mammalian cells, it is avirulent, because of the loss of two important host range genes among >25 mutations and deletions that occurred during its 570 serial passages through CEF [99, 100]. Despite its restricted host range and inability to produce infectious progeny in human cells, and in contrast to NYVAC (attenuated Copenhagen strain) and ALVAC (host range restricted avipox), both early and late transcription are unimpaired, making MVA a stronger vaccine candidate [101-104]. In fact, pre-clinical mouse studies have shown it to be more immunogenic than WR strain, and its ability to be used in conditions of pre-existing poxvirus immunity [105, 106]. Consistent with the ability to elicit responses against a recombinant MVA transgene product even in the face of pre-existing anti-vaccinia virus immunity, repeated administration of recombinant MVA allows re-boosting of responses, despite induction of cellular and humoral immune responses against the vector [107]. This has been reported both in Phase I/II therapeutic cancer vaccine trials in which up to 12 recombinant MVA vaccinations were given one month apart [108], as well as in a Phase I HIV vaccine trial in which up to three recombinant MVA vaccinations were given eight weeks apart [109].

Studies in rodents and macaques affirm the safety of MVA, including protection against more virulent forms of poxviruses in challenge models [110, 111]. After human inoculation, including high risk individuals, it is still avirulent without local or systemic reactivity under immunosuppressive conditions [104]. Similarly, a therapeutic vaccination with MVA expressing HIV-1 nef demonstrated its safety in HIV-1 infected individuals [49]. MVA immunization of malaria-infected adults or those challenged with attenuated malaria strains confirmed the safety of the vector and its ability to partially protect against a heterologous malaria strain [112, 113]. A trial in France and the USA showed that MVA expressing human MUC1 was safe and elicited T cell immunity when given to cancer patients [114]. Recent data from a study conducted at the Brigham and Women's Hospital (Boston, MA) by a team of investigators from Harvard Medical School and Dana-Farber Cancer Institute have shown that MVA was safe, well tolerated and immunogenic when used as a vaccine in HCT recipients[13]. An MVA expressing human p53 (p53MVA) developed and characterized at City of Hope [115] has been safely evaluated in a Phase 1 trial in advanced gastrointestinal cancer patients (IRB #10105)[116].

In animal disease models, recombinant MVA-based vaccines have either elicited systemic immunity or protection against influenza [117, 118], parainfluenza [119], RSV [120], dengue [121], Japanese encephalitis [122], malaria [123] and HIV/SIV [124-128]. Recently we used an MVA vaccine similar to that proposed in this study. This vector contained rhesus-CMV components and successfully protected CMV-negative macaques from infection [129, 130]. This data form the basis for development of CMV-MVA Triplex vaccine as a vaccine vector for CMV genes. This study will add to the current body of knowledge concerning safety and clinical efficacy of full-length antigens expressed in MVA.

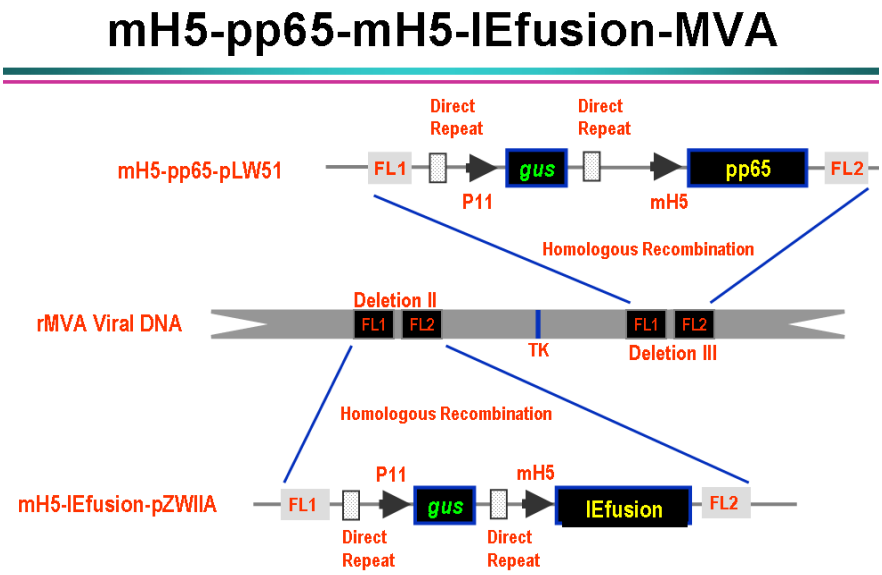
Construction, Expression and Function of 3-antigen MVA Vaccine: Choice of antigens encoded by CMV-MVA Triplex: 3 CMV gene products, UL83, UL122, and UL123 have been selected as targets for cell mediated immune (CMI) responses [61, 131-133]. [See Section 2.1.3 for a definition of these proteins.] UL83 has been shown by several groups to be the most immunogenic CMV structural protein (derived from the tegument) in terms of human CD8+ and CD4+ T cell responses [57-59, 134-137]. Recently, several reports have suggested that the CTL response to UL123 is as vigorous as the response to UL83 [31, 61-63, 138]. Each of the 3 antigens that are engineered into the multi-protein CMV-MVA Triplex vaccine are immunodominant, and their aggregate CMI recognition should exceed 95% of most ethnic populations [131]. All 3 antigens elicit memory CTL in humans that both lyse CMV-infected fibroblasts in vitro, and are quantifiable using HLA tetramers in fresh PBMC from CMV^+ individuals, thereby confirming their existence in vivo. A recent report suggests association of cellular immunity to UL83 and UL123 with recovery from CMV-retinitis in AIDS patients [139]. The accumulation of T cells specific for each of these 3 antigens in individuals with CMV reactivation episodes provides an additional justification for their inclusion in a vaccine [140]. Our recent monitoring of the immune response of a pre-clinical prototype of the CMV-MVA Triplex vaccine in the PBMC of HCT patients supports its use in transplantation [64]. The humoral response to proteins expressed from CMV-MVA Triplex vaccine is strong, but there is no evidence that the antibodies neutralize CMV [141]. The majority of the CMV-neutralizing antibody response has been localized to the gB (UL55) and UL128 gene products [129, 142-145]. As strong evidence that a humoral component impacts protection against CMV infection after HCT is lacking, gB has been omitted from this vaccine. This CMV-MVA Triplex vaccine strategy focuses on the CMI response that has been clearly demonstrated to be associated with protection from disease in HCT recipients.

Functional modification of CMV genes incorporated into MVA: A recent study of the immune response to full length UL83 in ALVAC and in TransVax™ DNA vaccine reported no short-term side effects (1-2 years) in both healthy volunteers and HCT patients [67, 93, 94]. Investigators have defined regulatory activity of the UL123 protein, including trans-activating properties on various cellular promoters [146-148]. Consequently 85 aa comprising coding exons 2 and 3 have been deleted. Deletion of the two coding exons results in a 406-aa protein that is no longer nucleus-associated, but cytoplasmic [61]. The 406 aa protein has minimal transactivation activity [147, 148]. Most known CTL epitopes from UL123 are found in exon 4, including the identified HLA A*0201-restricted CTL epitopes [62, 63, 138, 149]. Exon5 of UL122 was fused in frame to exon4 of UL123 without modification [64].

Insertion sites, promoter selection and permissive host cells for CMV-MVA Triplex vaccine generation: MVA was derived by serial transfer (570 passages) of the parental Ankara strain through chicken embryo fibroblasts (CEF) in order to derive a safe alternative to the smallpox vaccine[12]. As a result of its adaptation to CEF, several genomic deletions occurred [64, 99, 100]. These adaptations allow MVA to freely propagate in CEF to titers exceeding 10e10 pfu/mL, whereas standard mammalian cell lines such as CV-1 are non-permissive for propagation. For the pre-clinical studies conducted under GLP, specific pathogen-free (SPF) CEF, obtained from a qualified supplier (Charles River-SPAFAS), were used. As shown in Figure 1, deletion regions referred to as deletion II (del II) and III (del III) were used to insert foreign genes by homologous recombination [99]. To obtain the level of foreign protein expression that

results in a stable virus for manufacturing purposes, the modified H5 (mH5), promoter emphasizing early vaccinia gene expression has been utilized in MVA [152], and it provides a powerful boost to transcription of foreign gene inserts without causing genomic instability [51, 153] (Figure 2). The recombinant virus was made in two steps; the first being insertion of the pZWIIA plasmid, and the 2nd step was insertion of the of the pLW51 plasmid. The schematic representing construction of the MVA vector is shown below in Figure 1.

Figure 1: Construction of CMV-MVA Triplex vaccine



Construction of CMV-MVA Triplex vaccine with transient bacterial marker gene expression: the CMV-MVA Triplex vaccine was constucted using standard approaches of transfection-infection, purification of recombinant viruses and removal of the bacterial marker gene 3 HCMV genes were inserted into MVA using established homologous recombination methods [154]. An FDA-certified and traceable seed lot from a 1974 isolate was made available by B. Moss (NIAID) that pre-dates the bovine spongiform encephalopathy (BSE) outbreak in Europe. This seed lot is derived from the same batch that was approved by the FDA as a smallpox vaccine for use in the by Acambis/Baxter. Bacterial gene products that function as tracking markers in Viral or DNA vectors can cause stimulation of potent immune responses [155, 156]. The technology referred to as transient marker stabilization (TMS) which removes bacterial gene markers, was initially used to isolate of the CMV-MVA Triplex vaccine [157]. TMS allows the first screening to use a color substrate to distinguish the initial insertion as colored plaques. In subsequent rounds, the recombinants are rescreened by PCR and antibody staining to establish excision of the bacterial screening marker and presence of the CMV gene product respectively. Using this technology a new plasmid transfer vector (pZWIIA) was developed to enable construction of a marker-free CMV-MVA Triplex vaccine[158]. The 3-antigen CMV-MVA Triplex vaccine was constructed in three stages—the first being the fusion of UL123/e4 with UL122/e5, and verification of its expression and immune response [158]. The construction of the 3-antigen vector was furthered by inserting UL123/e4 - UL122/e5 under the control of the MH5 promoter in pZWIIA. CMV-pp65 was inserted into a derivative of pLW51 in which a single copy of the mH5 promoter drives the expression of the transgene. The final vector was extensively characterized for CMV antigen expression, absence of bacterial marker

sequences, immunogenicity in transgenic mouse strains, and a memory response in human PBMC [64, 159, 160].
The CMV-MVA Triplex has been tested in a phase I dose escalation study in healthy volunteers. Which has completed enrollment. The vaccine, administered twice in 28-day period, has shown marked safety in healthy adults vaccinated with up to $5x10^8$ pfu/mL. Additionally, CMV-MVA Triplex induces robust expansion of CMV-pp65, IE1 and IE2 CD8 and CD4 T cells in vaccinated research subjects. There have been no serious adverse events (SAE) or dose limiting toxicity (DLT) and few injection site reactions are the only notable AEs.

Since safety and immunogenicity have been established in healthy adults, there is a strong rationale to perform a Phase II study to assess safety and efficacy of CMV-MVA Triplex in protecting against CMV reactivation and disease in HCT patients at risk of life-threatening complications.

2.2 Overview and Rationale of Study Design

This randomized, blinded, placebo controlled, multi-site Phase II safety and efficacy trial will be conducted at COH, MDA and Dana-Farber. This study has been designed to have sufficient statistical power for testing clinically significant endpoints. In particular, the efficacy of CMV-MVA Triplex in protecting against CMV reactivation and disease in HLA-matched allogeneic (related or unrelated donor) HCT R^+ (CMV positive HCT recipients) who are at risk for CMV complications[18]. The primary hypothesis is that immunizing allogeneic HCT-R+ with CMV-MVA Triplex is safe and will provide superior protective benefit compared to a placebo. The secondary hypothesis is that CMV-MVA Triplex immunizations will induce protective levels of CMV cellular immunity in HCT-R+[161-163]. It is anticipated that the trial will provide definitive data on vaccine-induced protective immunity against CMV reactivation and disease in allogeneic HCT recipients [20].

Participants must be planned recipients of a first 8/8 HLA-matched[164-167] allogeneic HCT for hematological malignancies, CMV-positive, age 18-75 and willing to be monitored for 12 months following HCT. Exclusion criteria include receiving T cell depleted HCT, autoimmune disease, HIV, HCV and HBV positivity. Excluded diagnoses are aplastic anemia and multiple myeloma. Screening, enrollment and informed consent procedures will occur prior to the HCT procedure, as prospective participants are considered more able to make an informed decision at this time. Vaccine administration criteria will be assessed on the day of planned vaccination (Day 28 post-HCT). The Day-28-Post-HCT criteria exclude participants who post HCT have experienced disease relapse, CMV viremia or end organ disease, or have received anti-viral treatment or high dose steroids within 7 days of planned vaccine administration, or are experiencing ongoing grade 3 toxicities.

This phase II efficacy trial will enroll and vaccinate 102 allogeneic HCT-R^+, randomized to the vaccine (N=51) or placebo arm (N=51). Because approximately 10-15% of enrolled participants may fail to meet post-HCT vaccine administration criteria, the total study accrual is expected to be ~115 participants. A computer-generated 1:1 randomization stratified by donor CMV serostatus and center will assign participants to the CMV-MVA Triplex or placebo arms. The registrar located at the COH Data Coordinating Center will provide treatment assignment to the site pharmacists at COH, MDA or Dana-Farber, who are unmasked to treatment-group allocation. Because visual inspection of the vaccine at the time of administration may reveal the arm to which a participant has been assigned, persons administering the vaccine will receive training about the handling of the vaccine in front of the participant and colleagues. All study team members will receive orientation about the importance of maintaining a blinded randomization status. Participants meeting vaccine administration criteria will receive injections of either CMV-MVA Triplex or placebo on days 28 and 56 post-HCT, during the critical

period in which primary CMV reactivation most predictably occurs (~day 40-100)[28, 43]. Both the CMV-MVA Triplex and placebos are administered in a final 0.9-1mL volume, in the upper arm by intramuscular route.

Previous data, based on safety and immunogenicity in HCT recipients vaccinated with MVA support the use of $5x10^8$ pfu as the optimal dose[13]. Additionally, our data from the ongoing Phase Ib trial (COH IRB Protocol No. 08173) in healthy adults indicated that CMV-MVA Triplex was safe with limited injection site reactions, excellent tolerability and strong immunogenicity when 2 IM injections of $5x10^8$ pfu/mL were administered on Day 0 and Day 28. Thus based on this evidence, a dose of approximately $5x10^8$ pfu/mL CMV-MVA Triplex administered IM on day 28 and day 56 post-HCT was chosen for use in the HCT setting. Participants in the placebo arm will be injected with an isotonic solution of PBS containing 7.5% lactose, the diluent used in the CMV-MVA Triplex preparation. Participants who do not receive the first (Day 28) vaccine will be replaced and removed from the study. All participants who receive the first vaccine will be followed and assessed through Day 365 post-HCT, except for participants with demonstrated disease relapse. Participants with relapsed disease will be deemed ineligible upon diagnosis, and therefore will be followed for survival only to Day 365.

HCT recipients are closely observed for safety according to institutional standard of care practices, which recommend intense monitoring during the first 100 days following transplant. This study will follow the standard of care (SOC) practice till Day 100 post-HCT of weekly or biweekly assessments of CMV qPCR, clinical laboratory tests, engraftment assessment, GVHD assessment and physical exam. When clinically indicated, CMV disease and disease relapse will be assessed. Disease relapse will also be routinely assessed at pre-determined timepoints per SOC. The procedures and clinical care provided to participants will not differ from that provided routinely to all post-HCT patients with the exception of restricted concomitant medications that could impact study endpoints e.g. prophylactic antiviral treatment and T cell depleting agents.

Between Day 28 and Day 100, study visits will occur bi-weekly to coincide with vaccine administration and research blood sample collection, however information relevant to study endpoints can be obtained from any clinic visit. After Day 100, an additional four study visits will occur up to and including Day 365 to ensure periodic documentation and/or evaluation of clinical endpoints and for collection of research blood samples. The study will also investigate the impact of CMV-MVA Triplex on transplant related outcomes such as GVHD, disease relapse, mortality, and infection rate.

Rigorous stopping rules will be implemented, and will include three major safety endpoints:

- non-relapse mortality (NRM) at 100 days post HCT (Note: the rate of non-relapse mortality in the allogeneic hematopoetic cell transplant program at City of Hope and DFCI has been ≤10% and 12% respectively over the last five years).
- severe (grade 3-4) acute GVHD (aGVHD)
- grade 3-4 AEs (CTCAEv4) related to the vaccination within 2 weeks from each vaccination

CMV-specific immunogenicity (included in the secondary endpoints) will be evaluated in all participants in both arms (N=102) every 2 weeks from day 28 until day 100 post-HCT and on days 140, 180, 270, 365. Immunologic studies will include monitoring the levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated allogeneic HCT-R, combined with immunophenotyping studies [19, 21]. The phenotypic ratios of CMV-specific T cells will be related to improvement in control of CMV viremia. Based on the encouraging results of the Phase Ib (COH IRB 08173), levels of pp65, IE1 and IE2-specific T cells associated with protection from CMV viremia and disease are anticipated to be detected in a significantly higher proportion of vaccine recipients compared to those enrolled in the placebo arm[20]. Additional correlative immunogenicity studies will

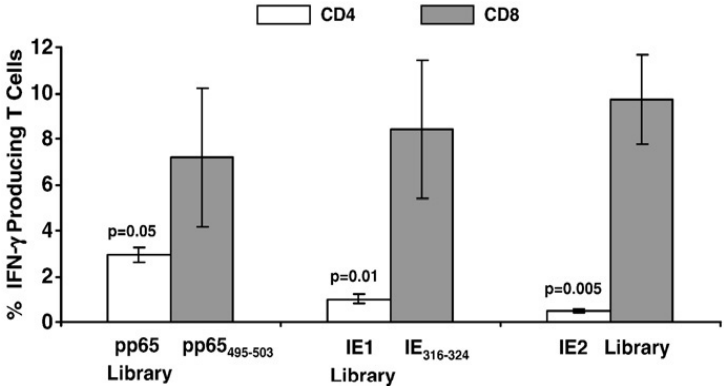
include measuring levels of highly cytotoxic memory NKG2C+ NK cells. These are linked to CMV reactivation, are critical for CMV adaptive immune responses and are potentially linked to relapse reduction[24]. To investigate the potential impact of CMV-MVA Triplex on GVHD, various GVHD biomarkers will be evaluated including: cytokines and hepatocyte growth factor for systemic GVHD [171, 172], elafin for skin GVHD [173], regenerating islet-derived 3α (REG3α) for gastrointestinal GVHD[174], suppression of tumorigenicity 2 (ST2) for steroid-refractoriness 5 [175] and CXCL9 [176, 177]and B cell–activating factor (BAFF) for chronic GVHD[176, 177]. The levels of these GVHD biomarkers will be compared between the vaccine group and placebo group, as well as between GVHD+ and – groups.

2.3 Preclinical Studies

2.3.1 CMV-MVA Triplex vaccine

Evaluation of the immunogenicity of the CMV-MVA Triplex in mouse models showed that the vaccine can stimulate primary immunity against all three CMV antigens (pp65, IE1 and IE2) in both the CD4 and CD8 T cell subsets. Since CMV-MVA Triplex activity is not HLA restricted, the vaccine has been successfully tested for CMV immune response in various transgenic HLA mouse strains including A2, B7, A1 and A11 [64, 160]. Intracellular cytokine secretion (ICS) methods were used to evaluate both CD4 and CD8 T cells responses to CMV-MVA Triplex vaccine using the peptide library approach applicable to any HLA type. We used commercially available pp65 and IE1 peptide libraries (PepMix^{TM}, JPT Peptide Technologies GmbH, Berlin, Germany)[72, 140, 178, 179]. An additional peptide library synthesized in our laboratory was used to detect responses against IE2 [64]. We were able to detect IFN-γ T cell responses using the peptide library approach [28, 64, 140]. Potency of the CMV-MVA Triplex vaccine were conclusively demonstrated in HLA transgenic mice [158], as shown in Figure 2.

A.



B.

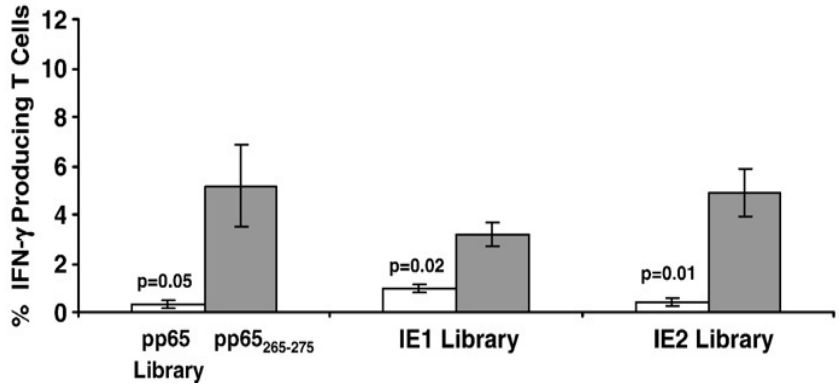


Figure 2: Potency of CMV-MVA Triplex vaccine in HLA transgenic mice

Immunogenicity of CMV-MVA Triplex vaccine in HLA A (A) and HLA B7 transgenic mice (B) as demonstrated by specific responses for pp65, IE1 and IE2 post immunization with 50 million pfu of vaccine. The % of IFN-γ producing $CD8^+$ T cells (grey bars) and $CD4^+$ T cells (unfilled bars) after stimulation with peptide epitopes or libraries are shown. Error bars represent standard error of the mean among the immunized mice (N=3). IFN-γ production in mock stimulation was subtracted in all experiments. P values indicate statistically significant differences.

2.3.2 Animal Toxicology

The persistence of vectored vaccines is a concern in clinical trials. Whilst the objective is to elicit immunity, side-effects due to long-term vector persistence and indefinite immune stimulation must be minimized. The non-recombinant form of MVA has been found to be safe in over 100,00 people [12, 95, 96] including HCT recipients [13]. However, the recombinant forms are unique and cannot be assumed to have an identical safety profile. Therefore pre-clinical toxicology studies of the cGMP-grade CMV-MVA Triplex Vaccine were conducted in rabbits at the Southern Research Institute (Birmingham, AL; Study # 13928.01.01). The vaccinated rabbits showed minimal toxicity, with the CMV-MVA Triplex vaccine being cleared from the injection site, blood and all other tissues within 46 days of administration.

2.4 Human Studies

CMV-MVA Triplex was evaluated *in vitro* for its ability to stimulate memory responses in PBMC from healthy adults and HCT recipients [64, 160]. Interestingly, CMV-MVA Triplex induced strong expansions of CMV-specific CD4+ and CD8+ T cell subsets in both healthy adults and patients within 6 months of receiving HCT. Based on the body of pre-clinical and *in vitro* results the FDA (BB-IND #15792) and IRB (08173) granted allowance to proceed with human studies. A Phase Ib clinical trial to evaluate the safety and biological efficacy of CMV-MVA Triplex vaccine in healthy volunteers, with or without prior immunity to CMV and vaccinia was initiated. This study indicated that CMV-MVA Triplex was safe and induced robust expansion of CMV-pp65, IE1, IE2, CD4 and CD8 T cells in 24 healthy volunteers (manuscript in preparation). This safety and immunogenicity data supports initiation of a Phase II trial in the HCT setting.

2.4.1 CMV-MVA Triplex stimulates CMV-specific T cells in human PBMC

We assessed the *in vitro* immunogenicity of CMV-MVA Triplex in healthy HCT donor and HCT patient blood specimens. CMV-MVA Triplex was first assessed in PBMC from healthy volunteers, providing a comparison with HCT patients, which are less well characterized. In both healthy individuals and patients there was brisk stimulation of antigen-specific T cell populations following *in vitro* stimulation(IVS) with CMV-MVA Triplex [64]. The memory T cell expansion stimulated by the CMV-MVA Triplex for pp65, IE1 and IE2 antigens followed the proportions found ex vivo with the same volunteers using the peptide library approach[72, 140, 178, 179]. Moreover they established that CMV-MVA Triplex stimulation does not substantially alter the relationship of the T cell subset proportion measured ex vivo for all three antigens. We found relationships among the T cell populations to be similar to published reports. pp65 promotes a substantial CD4 and CD8 response in over 70% of participants, while IE1 and IE2 are recognized less frequently and mainly in the CD8 T cell compartment[31, 63]. Interestingly, we found an equivalently strong recognition of CMV-MVA Triplex in HCT recipients, which in some cases was more vigorous than in the PBMC of healthy adults. Figure 3 shows the magnitude of the CMV-specific T cell responses in HCT patients. The T cell response was more pronounced in the CD8 population compared to the CD4 T cells, which is reflective of the ex vivo profile.

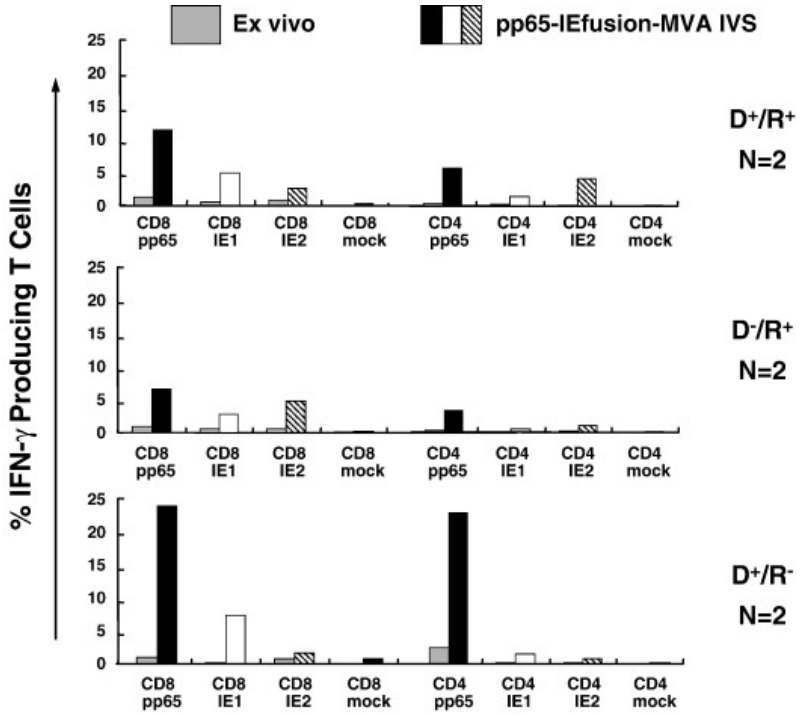


Figure 3: In vitro immunogenicity of CMV-MVA Triplex in HCT patients

CMV MVA Triplex stimulates CMV-specific T cells in PBMC from HCT recipients after IVS. A comparison was made between the ex vivo level (grey boxes) versus post-IVS for each stimulation. PBMC were divided into four aliquots and were individually co-incubated with pp65 (black), IE1 (white), IE2 (striped) peptide libraries or peptide diluent in single use aliquots. Standard gating procedures were employed for each individual flow acquisition, such that conditions were standardized for all evaluations. The plots show the percentage of IFN-γ producing T cells for each antigen-specific peptide library.

2.4.2 Phase Ib clinical trial in healthy adults (IRB 08173)

CMV-MVA Triplex vaccine was further evaluated in a Phase Ib clinical trial (IRB Clinical Protocol No. 08173), conducted in healthy adults to assess safety and immune response. To establish safety of CMV-MVA Triplex vaccine, a dose escalation study was performed in healthy volunteers (COH employees), ≥18 and ≤60 years of age, either CMV seropositive or seronegative. The study treated 8 subjects at 3 dose levels: $1x10^7$ pfu/dose, $5x10^7$ pfu/dose, and $5x10^8$ pfu/dose. Volunteers received two IM injections of 1mL volume in the upper non-dominant arm, each at the same dose over a 4-week period (see Fig 4).

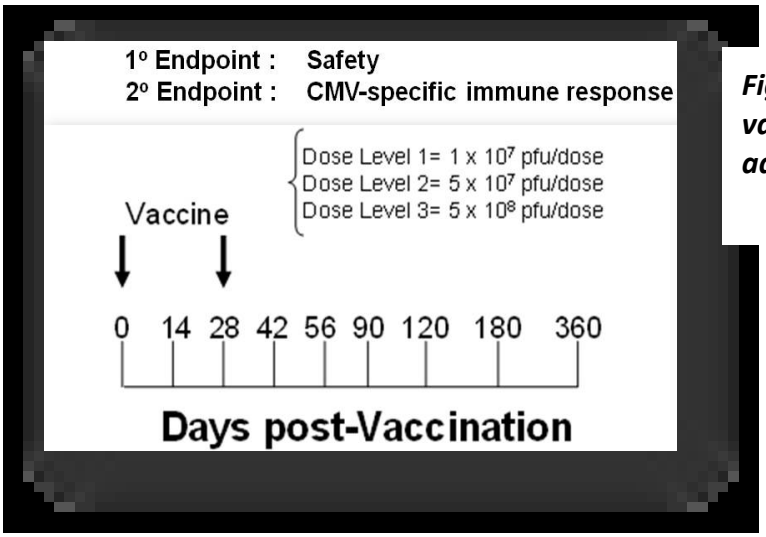


Figure 4: CMV-MVA Triplex vaccination schedule in healthy adults trial (Phase Ib)

2.4.2.1 Safety of CMV-MVA Triplex vaccine – primary endpoint

Among the twenty-four healthy volunteers (HV) vaccinated with CMV-MVA Triplex Vaccine at DL1, DL2 and DL3 few local site injections were reported. Enrollment has been completed, with none of the patients experiencing SAEs or DLT or withdrawing from the study. Thus, the first in human study of CMV-MVA Triplex vaccine has met the primary objective, showing an excellent safety profile (Table 1).

Report Date: May 14, 2015

Adverse Events Report Protocol 08173

Grade's frequency counts for adverse events assessed as possibly, probably, definitely related
For Toxicities Occuring Between: Activation and 05/14/2015
Title: Phase I Evaluation of a CMV-MVA Triplex Vaccine: Safety and Biologically Effective Dose in Healthy Volunteers With or Without Prior Immunity to CMV and Vaccinia

Attribution	Dose Level	Category	Adverse Event	Grade 1~2	Grade 3	Grade 4	Grade 5
Definite, Probable, Possible	**Arm 1 - Dose Level 1**		Cough (RESPIRATORY)	1			
			Headache (SYSTEMIC)	1			
			Hyperbilirubinemia (when accompanied by	1			
			Myalgia (MUSCULOSKELETAL)	2			
	Arm 2 - Dose Level 2		Fatigue (SYSTEMIC)	1			
			Headache (SYSTEMIC)	2			
			Induration (SKIN)	1			
			Myalgia (MUSCULOSKELETAL)	1			
	Arm 3 - Dose Level 3		Erythema (SKIN)		1		
			Fatigue (SYSTEMIC)	8			
			Headache (SYSTEMIC)	5			
			Hypertension (CARDIOVASCULAR)	1			
			Induration (SKIN)	1			
			Myalgia (MUSCULOSKELETAL)	7			
			Nausea (GASTROINTESTINAL)	2			
			Paresthesia (burning, tingling, etc.) (N	1			

Table 1: AEs in healthy adults trial (Phase Ib trial)

Adverse Events: Since rare occurrences of carditis with heart failure has occurred in human studies using a replicating vaccinia virus, the FDA recommended that all volunteers on this study be monitored for any change in electrocardiogram and cardiac troponin after vaccination[180]. No change was reported for all CMV-MVA Triplex vaccinated volunteers and no other cardiac AE were reported. Our results are in agreement with a recent systematic review, which reported cardiac safety surveillance from 6 Phase I trials of MVA vaccines. No detection of cardiac adverse reactions in any study participants immunized

with MVA was found. Unlike a replicating vaccinia virus, MVA is a highly attenuated and does not replicate in the human body[181]. A recent trial has shown that MVA was safe with no cardiac AE when used as a vaccine in HCT recipients, who were examined closely for vaccine induced cardiac side effects [13]. Additionally, an MVA expressing human p53 (p53MVA) developed by the Department of Experimental Therapeutics (DET)[115] was administered to 12 advanced gastrointestinal cancer patients in a Phase 1b study, with no SAEs observed (IRB #10105)[116]. *Persistence of Viral DNA*: Persistence of the CMV-MVA Triplex vaccine in healthy volunteers was monitored in accordance with FDA recommendations. If MVA DNA is detectable, monitoring in the study subject concerned is continued, up to a maximum of 12 months. The method used is based on an assay developed for assessing the presence of MVA sequences during the derivation of the vaccine at COH and was validated by the Quality Assurance Department of COH. This real-time PCR approach employs separate sets of primers for the MVA backbone and the CMV insert genes utilizing TaqMan™ reagents. The assay is sensitive to <20 copies of MVA DNA per sample and can be used to detect low level residual MVA in blood and other tissues. A plasmid DNA standard was employed to quantify the copy number of MVA and insert genes detected blood specimens. Blood and tissues from the CMV-MVA Triplex vaccinated rabbits (described in section 2.3.2) were tested in this way, and the results reported to the FDA as part of the BB-IND-13792 submission. From this successful submission, permission to proceed to the Phase I trial was granted. Persistence of the CMV-MVA Triplex vaccine was monitored in healthy volunteers after administration of both doses of vaccine, according to the methodology described above. Measurements were conducted in triplicate with one additional sample spiked with 50 copies of vaccine DNA. DNA from the dose level 1 and 2 cohorts showed undetectable levels of MVA DNA, or values below the detection limit of the assay. In the Dose Level 3 cohort, low levels of MVA DNA above the assay detection limit were detected at day 90, in 2 out of 8 of the vaccinated subjects. Since no MVA DNA was detected in these subjects blood at previous time points, these could be sporadic, anomalous results. Considering the high doses of CMV-MVA vaccine administered to these subjects, the low level of MVA DNA detected in these subjects, at a single time point, is not a cause for concern. Nonetheless, the day 360 time point will be monitored to confirm complete disappearance of MVA DNA.

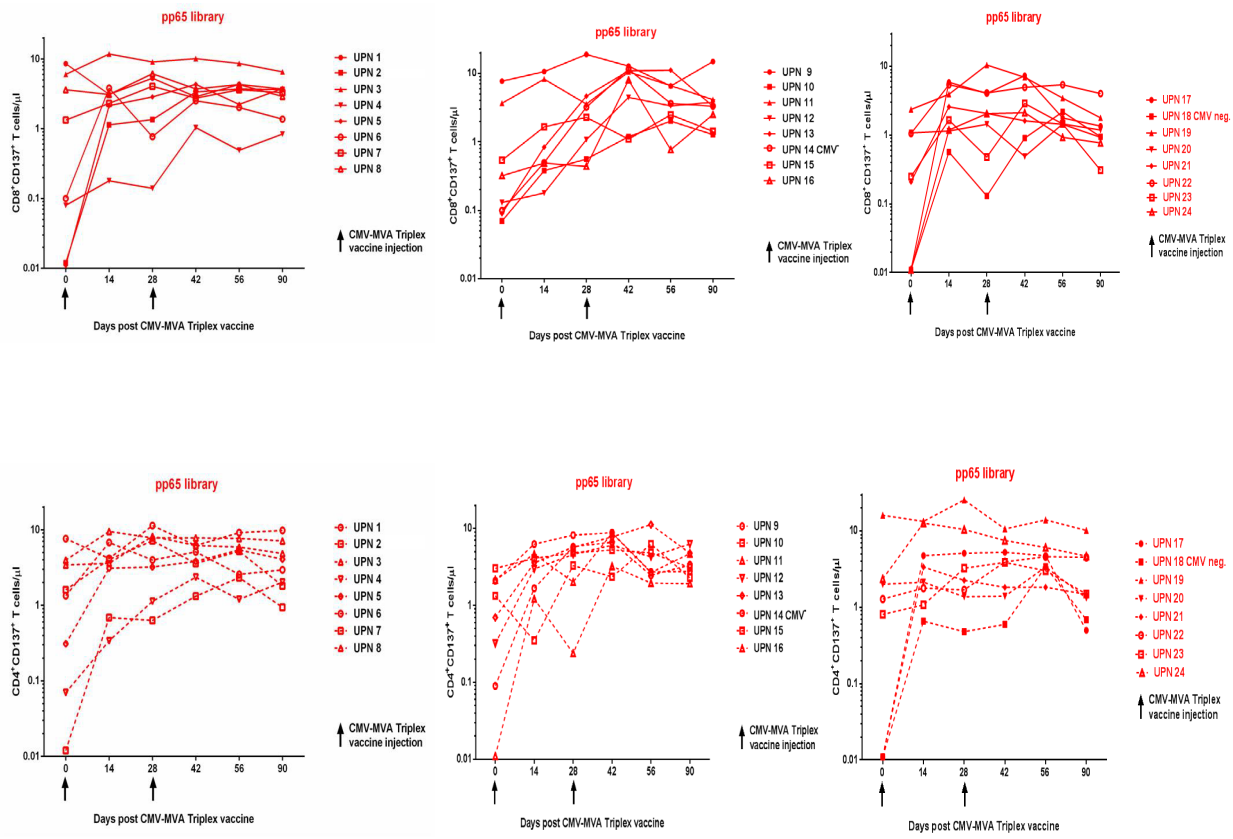
2.4.2.2 CMV-MVA Triplex Induced Immune Responses – secondary endpoint

CMV-MVA Triplex vaccine driven immune responses are robust in DL1 (N=8) and DL2 (N=8) healthy volunteers (Figure 5). Most immunized volunteers showed post-vaccination increases in CMV pp65, IE1- and IE2- specific T cell levels. The most significant values were observed in CMV positive subjects who had low levels of CMV-specific T cells pre-vaccination (Figure 5). We evaluated the cellular CMV-specific response by measuring CD137 on the surface of CD8 and CD4 T cells after 24 hours stimulation with pp65, IE-1 and IE2 peptides[182]. CD137 is expressed only on recently activated T cells, and its expression correlates with functional activation of T cells [183, 184]. We have previously shown that the frequency of CMV-specific T cells producing IFN-γ were lower than those expressing the CD137 marker [182, 184]. This suggests that T cell responses against CMV might not be fully characterized with a single functional cytokine assay. However, an antigen specific activation marker such as CD137, which is associated with multiple T cell functions, may provide a superior signal for CMV immune-monitoring in the transplant setting[185, 186]. Statistical comparisons (rank-sum test) indicate that average post-vaccination levels of pp65, IE1 or IE2 specific CD8 and CD4 T cells were significantly higher than baseline, with p-values ranging from 3×10^{-5} to 0.025. For example, pp65 stimulated CD4+ CD137+ T cells rose from a pre-vaccination median of 1.3 cells/μL to a post-vaccination median of 4.4 ($p = 3 \times 10^{-5}$) and pp65 stimulated CD8+ CD137+ T cells rose from a pre-vaccination median of 0.22 cells/μL to a post-vaccination median of 3.1 ($p = 0.003$).

Figure 5: Pre- and post-vaccination ex vivo CMV specific responses in HV immunized with CMV-MVA Triplex: *Levels of CD137⁺ CMV specific T cells in DL1 and DL2 cohorts. Each line shows the kinetics of the vaccine response with pre- and post vaccination levels of CD8 or CD4 T cells specific for pp65 IE1 and IE2 peptide libraries.*

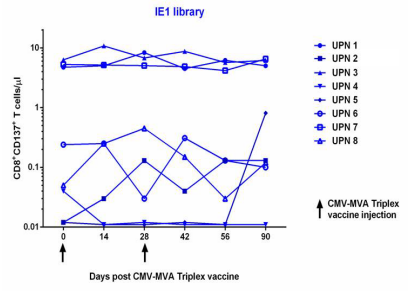
pp65

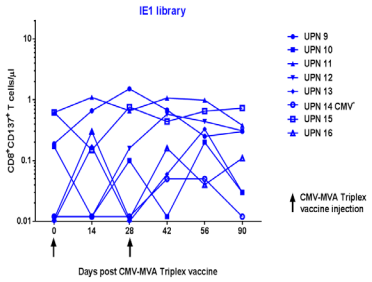
DL1 Summary **DL2 Summary** **DL3 Summary**

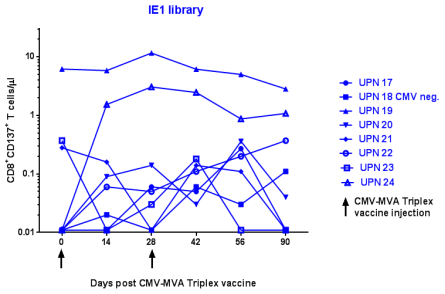


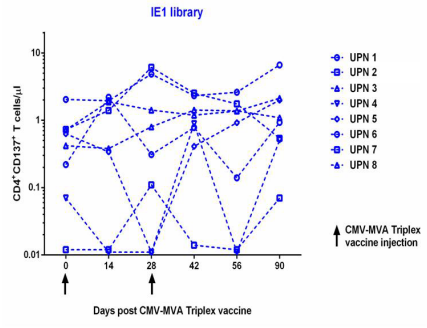
IE1

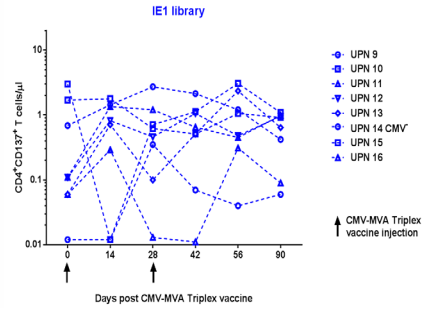
DL1 Summary **DL2 Summary** **DL3 Summary**

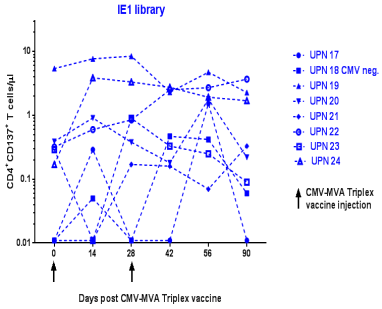




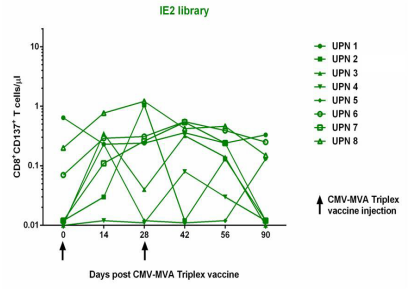


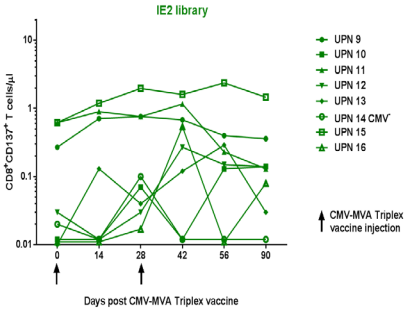


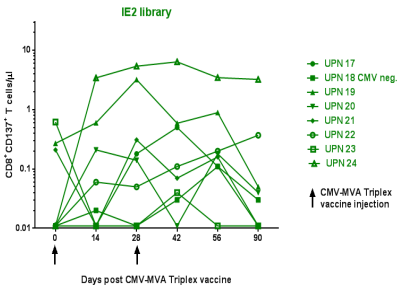


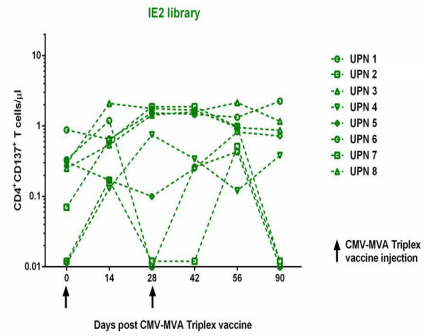


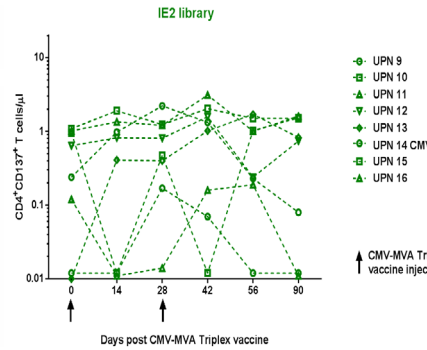
IE2

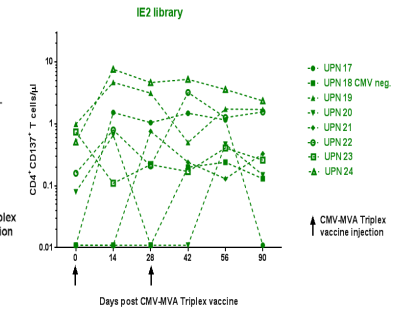












In addition, T cells specific for the immuno-dominant pp65$_{495-503}$ cytotoxic T cell epitope were monitored using Dextramer technology (Immudex, Copenhagen, Denmark), and were found to significantly increase post-vaccination. Interestingly, the response patterns to this epitope were similar to those detected against the whole pp65 peptide library (see Figure 6 below). These results are consistent with multiple findings showing the immune dominance of pp65 HLA A*0201 epitope and indicate that CMV-MVA Triplex does not alter the T cell recognition patterns of immunodominant CMV antigens measured ex vivo [15, 31, 52].

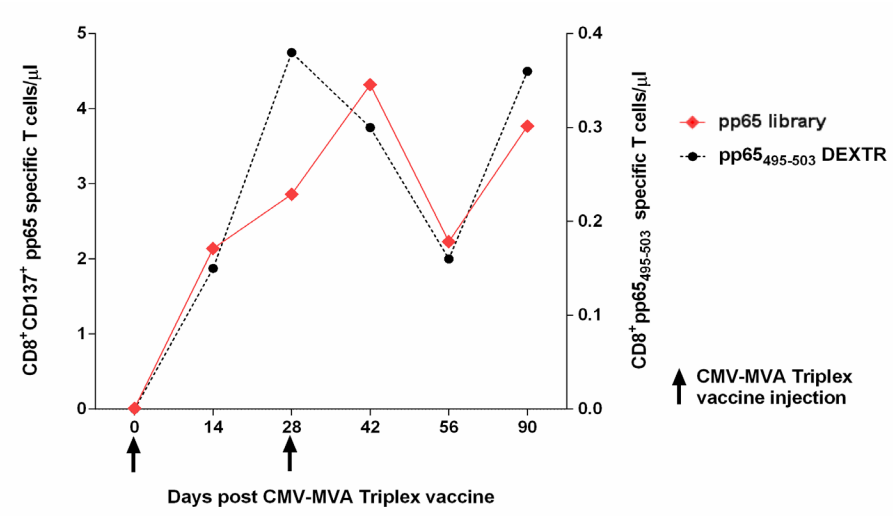


Figure 6: Ex vivo levels of pp65 specific T cell in an HV immunized with CMV-MVA Triplex:

*Pre- and post-vaccination levels of CD8 T cells specific for either the whole pp65 protein (left y axes, using pp65 library, red symbols) or the HLA A*0201 pp65$_{495-503}$ CTL epitope (right y axes, using pp65$_{495-503}$ Dextramers®, black symbols), in representative UPN 5 DL1.*

In conclusion, these preliminary results indicate CMV-MVA Triplex vaccine is strongly immunogenic in healthy adults even with the lowest dose of vaccine administered (10^7 pfu/mL), and that it does not alter the T cell recognition patterns from what has been found in naturally CMV infected healthy adults

Poxvirus Specific Immune Responses

All vaccinated volunteers were evaluated for poxvirus immunity after CMV-MVA Triplex vaccination. These evaluations were performed to assess the recognition levels of the MVA viral vector in healthy volunteers immunized with CMV-MVA Triplex vaccine.

Cellular immune responses

Vaccinia virus specific T cells levels were measured by CD137 assay [182] using peptide arrays composed of HLA supertype A and B epitopes of vaccinia virus proteins (obtained from NIH Biodefense and Emerging Infections Research Resources Repository, Manassas, VA). Post vaccination increases in vaccinia specific T cells were frequently detected in the peripheral blood of CMV-MVA Triplex vaccinated volunteers. Increased vaccinia virus-specific T cells were generally higher after the 2nd immunization and more conspicuous for CD8 than the CD4 T cell subset, as the vaccinia peptide arrays were mainly composed of CD8 T cell epitopes. Levels of vaccinia specific T cells vary among subjects. Interestingly, they were often of comparable magnitude to T cell levels elicited against the $pp65_{495\text{-}503}$ CTL epitope, but lower than the response to the whole CMV pp65 peptide library. Moreover they followed the kinetic patterns similar to those of the CMV immune responses (Figure 7).

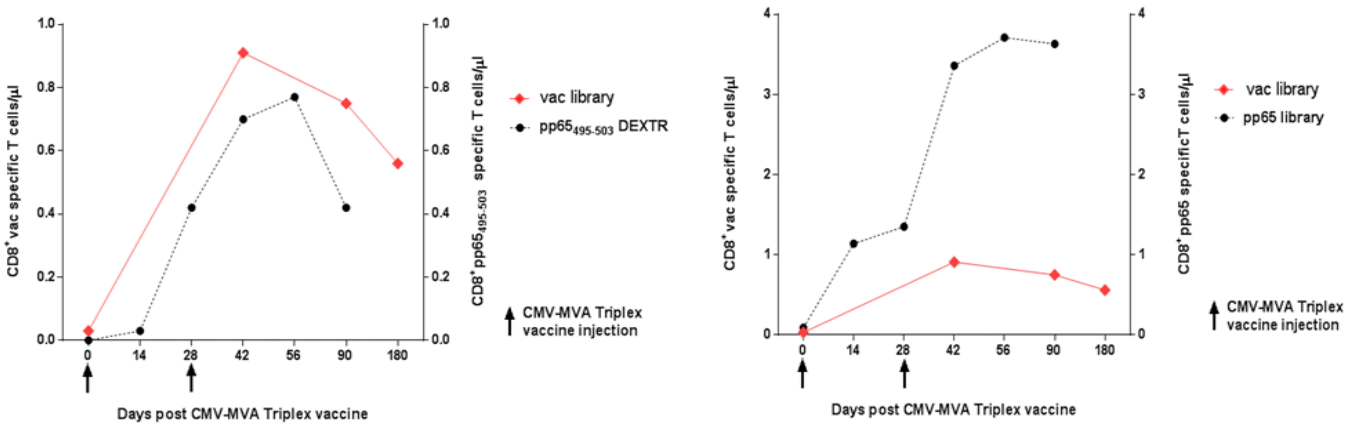


Figure 7 **Ex vivo levels of vaccinia and pp65 specific T cell in a healthy volunteer immunized with CMV-MVA Triplex:** *Pre- and post-vaccination levels of CD8 T cells specific for the vaccinia peptide library (red line) compared with levels specific for the $pp65_{495\text{-}503}$ CTL epitope (left panel, black dotted line) and the whole pp65 peptide library (right panel, black dotted line) in representative UPN 2 DL1.*

In the DL1 vaccine cohort four volunteers were born before 1973, while the remaining subjects were born on or after 1973. 1973 was the final year of smallpox immunization in the U.S. [187] and these four subjects had received smallpox vaccination. However, their previous immunity with vaccinia did not result in marked differences in the T cell responses compared to vaccinia naïve subjects (see Figure 8). Our results are in agreement with previous longitudinal studies in which antiviral antibody responses were found to be highly stable and could be present 1 to 75 years post-vaccination, while anti-vaccinia T cell responses decreased 8 to 15 years after the last vaccination[187, 188].

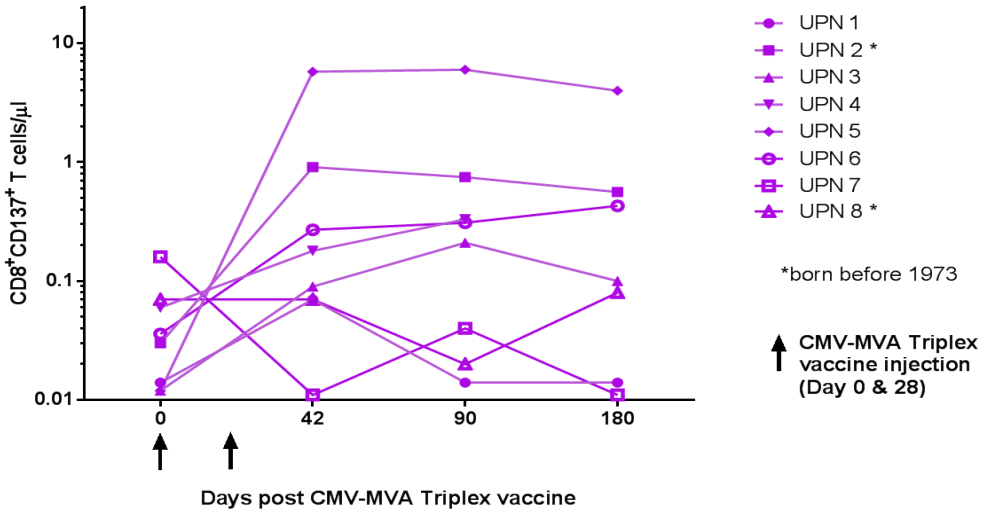


Figure 8: Ex vivo levels of vaccinia specific T cells in CMV-MVA Triplex vacinees with or without prior vaccinia immunity *Pre- and post-vaccination levels of CD8 T cells specific for the vaccinia peptide library in 8 heathly volunteers, including UPN 5 who was vaccinia naïve at the time of* Triplex vaccination *(year of birth 1990)* and UPN 8 *who had previously received smallpox vaccination (year of birth 1968).*

Humoral response

Vaccinia neutralization assays were performed with a modification of a published protocol[189] using an MVA expressing the fluorescent marker Venus [129]. Briefly, serial 5-fold dilutions of sera, starting with a 1:20 dilution, were incubated with 0.5×10^6 pfu of venus fluorescent-expressing vaccinia virus. Subsequently human B-lymphoblastoid cell lines (LCL) were added at a concentration of 0.5 pfu per cell in 96-well plates. Fluorescence intensity in infected cells was compared with wells containing cells and virus, but no serum (positive control) and cells and serum containing no virus (background control). In addition, anti-rabbit vaccinia purified serum anti-vaccinia rabbit sera (a kind gift from Dr. Mary Marovich, Henry Jackson Foundation, Bethesda, MD) was used as an internal reference standard. Neutralization antibodies specific for vaccinia virus were raised in the large majority of the volunteers immunized with CMV-MVA Triplex vaccine. Levels rose after the second injection and remained stable until the study was concluded. As expected, all 4 study volunteers from DL2 who were born before year 1973 did have detectable vaccine virus neutralizing antibody before vaccination with CMV-MVA Triplex vaccine (Figure 9).

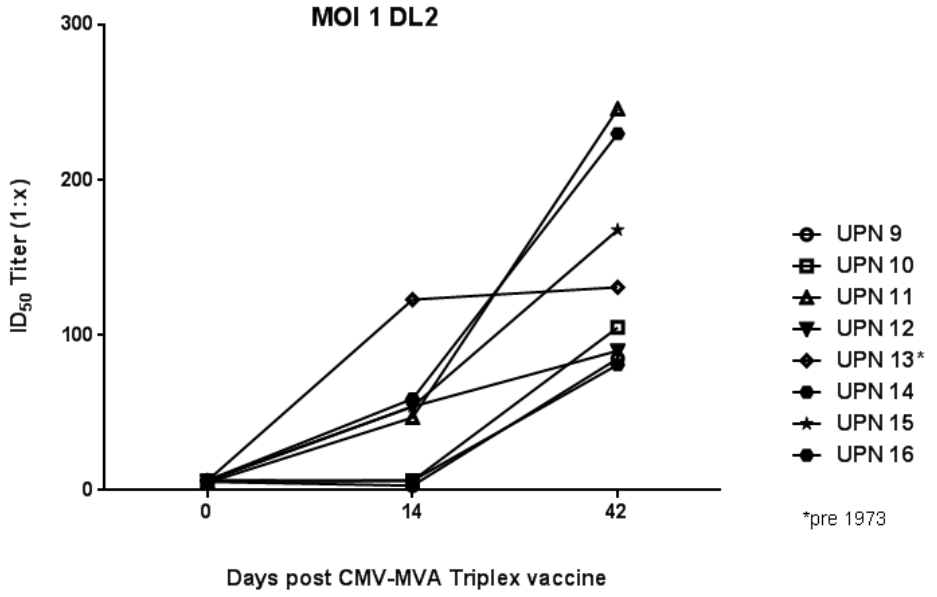


Figure 9: Levels of vaccinia virus neutralizing antibodies in CMV-MVA Triplex vaccine immunized subjectsfrom the DL2 cohort

The ID50 titer was calculated as the serum dilution that caused a 50% reduction in venus expression (green fluorescence) on the vaccinia-infected LCL compared to the virus+LCL control samples after subtraction of uninfected LCL control. A positive response for the CMV-MVA Triplex vaccine *was defined as a titer ≥ 2 times the baseline (day 0) titer and ≥ 1:20.*

3.0 PARTICIPANT ELIGIBILITY CRITERIA

3.1 Pre-HCT Inclusion Criteria

Participants must meet all of the following criteria on screening examination to be eligible to participate in the study:

Informed Consent and Willingness to Participate

___ 1. All subjects must have the ability to understand and the willingness to sign a written informed consent.

___ 2. Participant must be willing to comply with study and/or follow-up procedures, including willingness to be followed for one year post-HCT.

Age Criteria

___ 3. Age 18 to 75 years.

Nature of Illness and Transplant Related Criteria

___ 4. Planned HCT for the treatment of the following hematologic malignancies:

- Lymphoma (Hodgkin and Non-Hodgkin)
- Myelodysplastic syndrome
- Acute lymphoblastic leukemia in first or second remission (For Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma, the disease status must be in hematologic remission by bone marrow and peripheral blood. Persistent lymphadenopathy on CT or CT/PET scan without progression is allowed.)
- Acute myeloid leukemia in first or second remission
- Chronic myelogenous leukemia in first chronic or accelerated phase, or in second chronic phase
- Other hematologic malignancies including chronic lymphocytic leukemia, myeloproliferative disorders and myelofibrosis. Patients with multiple myeloma and those with non-malignant disease such as aplastic anemia are excluded*

*Adult cases of multiple myeloma (MM) are excluded as allogeneic HCT is not standard of care for MM, and is only performed in very advanced cases with an associated high risk of relapse and NRM. Adults with aplastic anemia are excluded because their standard management includes T cell depletion with agents such as ATG, which is not permissible on this protocol (see Section 3.2 point 6). Patients undergoing a second allo HCT are not eligible (patients who have undergone a previous autologous HCT are eligible).

___ 5. CMV seropositive (recipient)

___ 6. Planned related or unrelated HCT, with 8/8 (A,B,C,DRB1) high/intermediate resolution HLA donor allele matching

___ 7. Planned HCT with minimal to no-T cell depletion of graft

___ 8. Conditioning and immunosuppressive regimens according to institutional guidelines are permitted

Clinical laboratory parameters

___ 9. Negative serum or urine β-HCG test (female patient of childbearing potential only) within two weeks of registration.

___ 10. Seronegative for HIV, HCV and active HBV (Surface Antigen Negative) within 2 months of registration.

Child Bearing Potential

___ 11. Agreement by females of childbearing potential **and** sexually active males to use an effective method of contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for up to 90 days post-HCT. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

3.2 Pre-HCT Exclusion Criteria

Prospective participants who meet any of the following criteria will not be eligible for admission into the study:

Previous therapies

___ 1. Any prior investigational CMV vaccine

___ 2. Experimental anti-CMV chemotherapy in the last 6 months

Planned medications from the time of HCT to day 70 post-HCT

___ 3. Live attenuated vaccines

___ 4. Medically indicated subunit (Engerix-B for HBV; Gardasil for HPV) or killed vaccines (e.g. influenza, pneumococcal, or allergy treatment with antigen injections)

___ 5. Allergy treatment with antigens injections

___ 6. Alemtuzumab or any equivalent in vivo T-cell depleting agent

___ 7. Antiviral medications with known therapeutic effects on CMV such as GCV/VAL, FOS, Cidofovir, CMX-001, maribavir. Acyclovir has no known therapeutic efficacy against CMV and is allowable as standard of care to prevent HSV.

___ 8. Prophylactic therapy with CMV immunoglobulin or prophylactic antiviral CMV treatment

___ 9. Other investigational product – concurrent enrollment in other clinical trials using any IND drugs with unknown effects on CMV or with unknown toxicity profiles is prohibited.

___ 10. Other medications that might interfere with the evaluation of the investigational product (see Prohibited Medications, Section 5.6)

Other illnesses or conditions

___ 11. Patients with active autoimmune conditions requiring systemic immunosuppressive therapy within the previous 5 years are not eligible

___ 12. Pregnant women and women who are lactating. CMV-MVA Triplex risks to pregnant women are unknown. Because there is an unknown but potential risk for adverse

events in nursing infants secondary to treatment of the mother with the administered vaccine, also breastfeeding should be discontinued if the mother is enrolled on this study.

___ 13. Any other condition that would, in the Investigator's judgment, contraindicate the patient's participation in the clinical study due to safety concerns or compliance with clinical study procedures, e.g., social/ psychological issues, etc.

Noncompliance

___ 14. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

3.3 Participation of Special Populations

A discussion of the inclusion, exclusion, and representation participation of women, minorities, children and HIV positive individuals is provided in Section 16.5.

4.0 PARTICIPANT ENROLLMENT AND RANDOMIZATION

4.1 Pre-Enrollment Informed Consent and Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility will be done only after obtaining written informed consent. Studies or procedures that are performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values and/or to determine pre-eligibility, even if the studies were done before informed consent was obtained. The informed consent process is to be fully documented, and the prospective participant must receive a copy of the signed informed consent document. See Table 10 footnote f and Section 16.4 for more information regarding informed consent. Screening procedures are listed in Section 10, Table 10 Study Activity Calendar.

4.2 Participant registration

4.2.1 COH DCC Availability and Contact Information

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope. DCC staff are available **between the hours of 8:00 a.m. and 5:00 p.m. PST, Monday through Friday (except holidays).** DCC contact information is as follows:

- phone: (626) 256-4673 ext. 63968
- e-mail: DCC@coh.org

4.2.2 Slot verification and reservation

As the study nears completion of accrual, study team personnel (including physicians, protocol nurses and/or CRCs) may wish to contact the DCC to verify slot availability and to reserve an open slot or be placed in queue for slot opening. Slots may only be held for a limited time which will be determined by the PMT. The Data Coordinating Center should be notified of cancellations of prospective participants holding slots as soon as possible.

4.2.3 Registration procedure

To register a participant, the subsequent procedure is to be followed.

1. The participating site's data manager/coordinator/research nurse should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window (60 days to 0 days before planned HCT).
2. The data manager/coordinator/research nurse should then e-mail copies to DCC@coh.org of the following documents to the DCC:
   - Registration Cover Sheet (Appendix F or G)
   - Completed Eligibility Criteria List
   - Source documentation to support eligibility criteria**
   - Signed informed consent document (if permitted by institutional policy)
   - Signed HIPAA authorization form (if separate from the informed consent document)
   - Signed subject's Bill of Rights (COH only)

   **For COH participants, provide copies of source documentation only if not readily available as a finalized record in the COH EMR
3. After having received all transferred documentation, the DCC will review the documents to verify eligibility, working with the participating site as needed to resolve any missing required source elements. A subject failing to meet all protocol eligibility requirements will not be registered.
4. Once eligibility has been confirmed, DCC staff will register the participant by: assigning a subject accession number, register the subject on study centrally into MIDAS for MD Anderson and Dana-Farber participants (the COH CRC will directly accession into MIDAS), and enter the subject into the eCRF system, Medidata RAVE.
5. Once registration has been completed, DCC staff will send a Confirmation of Registration Form, including the participant study number and planned date of HCT procedure to:
   - the site study team: site PI, treating physician, protocol nurse, CRC and pharmacy
   - the sponsor team: (Drs. Nakamura, Aldoss, La Rosa, and the COH CRC)

4.3 Randomization

The treatment assignment will be masked from patients and health care providers, but known to the DCC coordinators, study monitors, auditors, pharmacy personnel, and the statistician. See Section 5.2 for additional information about maintaining a blinded randomization.

The DCC staff will use a computer-generated randomization , stratified by donor CMV serostatus and center to assign registered participants to the CMV-MVA Triplex or placebo arm. The treatment assignment will be generated and provided to pharmacists in advance (usually ~day 21 post-HCT) of planned vaccination. The DCC staff will confirm that the treatment assignment was received by the pharmacy.

The DCC specialist will request a copy of source documents regarding donor CMV serostatus, if not already provided/available. The DCC staff may contact the study team coordinator to confirm the HCT procedure did occur prior to generating the treatment assignment.

As soon as the study team is aware that a registered participant will NOT meet the criteria to receive the initial vaccine, this should be promptly communicated to the DCC. This information will be entered into the computer-generated randomization program to inform subsequent treatment assignments.

Only participants who receive a vaccination (CMV-MVA Triplex or placebo) on day 28 will be considered "randomized".

4.4 Emergency De-Blinding Procedures

Participant's randomization status will be un-blinded in the event a patient on this study develops a life-threatening toxicity or serious adverse event for which the participant's physician or other health care professional feels that it is in the patient's best interest to know the randomization status of the participant.

The following procedure should be followed:

Information regarding the rationale for de-blinding will be provided to the Site PI, the COH DCC, and the Study PI.

The Site PI will communicate with Pharmacy and will provide authorization to un-blind patient's treatment.

In this very unlikely event, the PMT will determine if and how the de-blinding should impact the participant's continued participation in the study or analysis of collection points post de-blinding. This plan will be provided to the IRB of record and the COH IRB and DSMC as per COH institutional requirements. The date and reason for de-blinding must be noted in the medical record and captured in the eCRF.

5.0 TREATMENT PROGRAM

5.1 Treatment Overview

The study intervention will consist of a vaccine administration of either CMV-MVA Triplex (N=51) or placebo (N=51), depending on participant randomization.

Participants will receive vaccine administration on days 28 and 56 post-HCT if vaccine administration criteria are met (Sections 6.1 and 6.2) and confirmed by the DCC (who will notify the study team pharmacy of the confirmation).

Participants who do not receive the Day 28 Post HCT vaccine administration will be replaced. Participants who receive at least one vaccine administration will complete all procedures detailed in Section 10, except for participants who experience relapsed disease for whom all post-relapse assessments will cease and will be followed only for survival through day 365-Post-HCT.

Windows for vaccine administration are detailed in Section 10, Study Activity Calendar.

5.2 Maintaining a blinded randomization

The treatment assignment will be masked from patients and health care providers, but known to the DCC coordinators, study monitors, auditors, pharmacy personnel, and the statistician. Due to differences in appearance of the active agent and the placebo, the person administering the vaccine (placebo or active agent) should limit discussions regarding the characteristics regarding the agent's appearance or

administration (i.e. ease or difficulty of administration) to the study pharmacist. Since the person administering the vaccine may detect a difference in appearance between placebo or active agent, he/she will be furthermore excluded from performing any protocol-required procedures or providing day-to-day medical care of the participant for the duration of the trial. The pharmacist will keep the treatment assignments and accountability documentation such that the documents cannot be accessed by the individuals who conduct protocol-required assessments, follow-up assessments, or those involved in the day-to-day medical care of the subjects during the trial. Nicola Hardwick will be the designated un-blinded PMT contact for Pharmacy. Disclosure of any knowledge of the randomization status to persons other than those permitted to know the randomization status would result in a protocol violation.

5.3 Assessments

Patients undergoing HCT are heavily monitored for safety according to institutional SOC practices. The following assessments will occur for safety and/or endpoint analysis with the schedule indicated in Study Activity Calendar (Section 10), per institutional SOC, and as clinically indicated.

5.3.1 Post-Vaccination Assessment

Vaccine and placebo injections will be administered intramuscularly. All subjects will be monitored for at least 30 minutes after each immunization for any local or systemic reactions including vital signs (temperature, pulse, blood pressure, and respiratory rate). Notation will be made of the subject's temperature and of any local reaction at the injection site.

5.3.2 GVHD assessment and performance status

Acute GVHD will be assessed and graded according to the Keystone Consensus grading system (Appendix A). Chronic GVHD will be classified per Appendix B by type of onset (progressive, interrupted, de novo, or chronic); basis of diagnosis (histologic/biopsy proven, clinical evidence, both, or unknown); Limited or Extensive chronic GVHD; and overall severity of GVHD (mild, moderate, or severe). Performance status will be evaluated utilizing the Karnofsky Performance Scale (Appendix D).

5.3.3 CMV monitoring

For CMV monitoring, standard qPCR clinical laboratory methods will be used to evaluate CMV viral load and possible vaccine failure at least weekly (usually twice weekly), or as required by SOC until day 100. The methods used at the two study sites are as follows:

COH: Focus 3M Integrated Cycler and Simplexa CMV Kit for *in vitro* diagnostic use, detection limit 250 gc/ml, reported in WHO IU/ml (conversion factor 2.5; 250gc/ml=625IU/ml, note all PCR values in this protocol are expressed by gc/ml based on the COH assay).

DFCI: Roche Cobas CMV VL assay, detection limit 150 gc/ml, one copy equivalent to 0.91 WHO International Units (IU).

MDA: Cobas CMV PCR, detection limit 97 CMV IU/mL, with a reportable range >137 IU/mL.

Based on available information these methods are considered sufficiently similar. However, selected samples from DFCI study subjects will be stored for batch testing at COH to confirm equivalence of the two methods (see study calendar for details).

Clinical CMV disease status will be documented at each study visit, which may include the absence or presence of suspected CMV disease. When clinically indicated and per SOC, CMV disease will be assessed and, when present, the site (upper GI, lower GI, other, specify) and method of detection in the tissue (tissue culture, pathology etc.) will be documented. Presentations or suspected presentations of

CMV disease in the absence of qPCR >500 gc/mL will be evaluated by the treating investigator in conjunction with the blinded PMT before a determination is made.

5.3.4 Engraftment assessment

Engraftment will be assessed by monitoring the recipient's absolute neutrophil count. The date of engraftment is defined as the first date of 3 consecutive laboratory values obtained on different days when the peripheral blood absolute neutrophil count is ≥ 500/mm^3; for the purposes of recording into the case report form, the date of engraftment can be derived from the ANC values in the clinical laboratory results so long as there is accompanying documentation in the medical record that engraftment did occur.

Graft failure following engraftment (secondary graft failure) is defined as a fall in the absolute neutrophil count below 500/mm^3 for 3 or more consecutive laboratory values following initial engraftment that is not due to disease relapse/progression, infection or secondary medication effect; the date of graft failure will be defined as the date when the criteria for graft failure are confirmed by the clinician-investigator.

5.3.5 Disease relapse

Disease relapse will be assessed (including timing of assessment) according to institutional SOC practice for the participant's specific hematologic malignancy. At defined clinic visits, the disease relapse status should be documented, which may include the presence or absence of clinical signs of disease relapse. Relapse is defined as "morphologic relapse" for acute/chronic leukemia including AML, ALL, MDS, CML. Patients who are found to be in relapse only by molecular methods or cytogenetics will continue with the protocol therapy, except for cases which received conventional cytotoxic chemotherapy. The following FDA-approved tyrosine kinase inhibitors/hypomethylating agents are allowed in case of molecular/cytogenetic relapse: imatinib, nilotinib, dasatinib, ponatinib, sorafenib, azacitidine and decitabine. Participants who undergo disease relapse will cease all future study visits/procedures and will be followed only for survival through Day 365.

5.3.6 Clinical laboratory chemistry, hematology, EKG and pregnancy test

A complete metabolic panel will include the following 18 blood chemistry parameters (CMP): glucose, BUN (blood urea nitrogen), creatinine, uric acid, total proteins, albumin, calcium, phosphorous, sodium, potassium, chlorine, total CO_2, total bilirubin, alkaline phosphatase, ALT (alanine transaminase), AST (aspartate aminotransferase), LDH (lactate dehydrogenase), total cholesterol.

An EKG will be performed at days 47 and 77 (+/- 10 days) to identify any vaccine related cardiac AEs if the patient is in a state of health that can tolerate the assessment.

For women of child bearing potential, a serum or urine pregnancy test is acceptable. Female participants who are menopausal are not considered of child bearing potential. Menopausal status is defined as more than 12 months without menses, or a medical history of menopausal status (estrogen level and follicle stimulating hormone in the menopausal range).

The hematology testing will include a complete blood count (CBC) with differential.

5.3.7 Adverse event assessment (CTCAE)

All adverse events will be assessed using NCI CTCAE v. 4.0, which can be found at the following link: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf8.1. Adverse events recorded in the source documents and the case report forms include:

- All events considered possibly, probably or definitely related to study agent till end of the study period (Day 365)
- All grade 1 and 2 events that occur within 2 weeks from each vaccination
- All grade 3/4/5 events will be reported from Day 28 through Day 100
- All serious adverse events will be reported to the sponsor from Day 28 through Day 100
- After Day 100, all deaths will only be reported in the follow-up visit page of the eCRF.

5.3.8 Physical exam, vital signs, medical history, baseline symptoms, and demographics

Physical exam including a review of skin will be performed. Vital signs will include review of weight, heart rate, blood pressure, respiration rate, and temperature. Height will only be required at baseline.

Medical history is defined as any significant medical conditions up to Day 0, but prior to stem cell infusion. Baseline symptoms are defined as any symptoms on Day 28 prior to vaccination.

5.3.9 Concomitant medications

All medications, supportive care, blood products or radiation therapy taken or administered during the trial will be documented in the subject's clinical/hospital record, using COH, MDA and Dana-Farber documentation guidelines. Medications related to a serious adverse event will be collected in the study case report forms as appropriate:

- Concomitant medications
- Anti-viral medications, including indication, start and stop date
- Immunosuppressive agents
- Prednisone, or equivalent, dosage for the 7 days prior to vaccine administration
- Prohibited medications

5.3.10 Immunogenicity testing

All participants will undergo serial blood sampling for future immunogenicity testing. Section 9.0 details regarding sample collection, storage, and processing procedures for immunogenicity studies.

5.4 Criteria for Completing/Discontinuing Study Participation

Participation may continue until one of the following criteria applies:

- Participant does not meet criteria for Day 28 vaccine (such participant are not randomized and will be replaced)
- Completion of study procedures
- Participant withdraws from the study
- General or specific changes in the participant's condition that render the participant unacceptable for participation in the opinion of the treating investigator.

Documentation of the reason for completing study participation and the date effective should be made in the medical record and appropriate eCRF. The COH DCC should be promptly notified of the change in participant status.

5.5 Follow-Up and Duration of Participation

The length and involvement of study participation will vary based on vaccine administration, disease relapse, or decision to withdraw from the study, as detailed in the subsections that follow:

5.5.1 Participants who do not receive Day 28 vaccine administration

Participants who do not meet criteria for Day 28 vaccine will discontinue any further follow-up.

5.5.2 Participants who relapse after receiving vaccine administration

Participants who relapse after receiving vaccine administration will have procedures performed and documented up to that time of determination of relapse, and then will be **followed for survival only** (including reason for mortality) until Day 365 post-HCT.

5.5.3 Participants who withdraw from the study after receiving a vaccine administration

Participants who withdraw from the study after receiving a vaccine administration may continue with follow-up per the participant's agreement:

- may elect to continue study monitoring procedures without Day 56 vaccine (if not already administered),
- may elect to continue to be monitored for survival (including reason for mortality) until Day 365 only, or
- may elect to withdraw completely; further follow-up or assessments will not occur.

5.5.4 All other participants (vaccine administered, have not relapsed or withdrawn)

All participants who received at least one vaccine, have yet to have disease relapse, and have not withdrawn from the study, will continue follow up assessments and research blood draws as indicated in the Study Activity Calendar (Section 10) through day 365 post HCT. Study participation will be completed on Day 365 Post-HCT.

5.6 Supportive Care, Other Concomitant Therapy, Prohibited Medications

In general, the use of any concomitant medication/therapies and supportive care deemed necessary/appropriate for the care of the participant are allowed, with the following exceptions:

- No other investigational agents may be given to patients
- Alemtuzumab or any equivalent in vivo T-cell depleting agent is not permitted in this study following HCT, because its administration results in *in vivo* depletion of B, T and dendritic cells, potentially negating any positive effect of vaccinating the recipient with CMV-MVA Triplex.
- Preemptive therapy with CMV immunoglobulin or antivirals (GCV/VAL, FOS, Cidofovir, CMX-001) is not allowed following HCT. GCV/VAL, FOS, Cidofovir, CMX-001 may be used according to institutional SOC for preemptive management of CMV viremia. In general, therapy should not commence until after CMV qPCR ≥500 gc/mL. For preemptive therapy when qPCR<500, the study PIs are to be consulted.
- Prophylactic antiviral treatment for HSV, HHV6, EBV and adenovirus including the use of GCV/VAL, FOS, Cidofovir, CMX-001 may also suppress reactivation of CMV, thus will not be allowed in this study following HCT. Therapeutic use of these agents is permitted per institutional standard practice.. Acyclovir has no therapeutic efficacy against CMV and is allowed as standard of care to prevent HSV.

- Medications that might interfere with the evaluation of the investigational product are prohibited up to 14 days after the second vaccination (day 70 post-HCT). Medications in this category include, but are not limited to:
    - Live attenuated vaccines
    - Medically indicated subunit (Engerix-B for HBV; Gardasil for HPV) or killed vaccines (e.g. influenza, pneumococcal, or allergy treatment with antigen injections)
- Steroid therapy with prednisone, or equivalent, is permitted (see Section 6.0 for specific steroid dose criteria for vaccinations).

6.0 VACCINE ADMINISTRATION CRITERIA

6.1 Day 28-Post-HCT Vaccine Administration Criteria

In instances where the criteria are not clear cut, the treating clinician and both Site PIs will collaborate to make a determination. This consultation should be documented.

Following randomization, a first injection (Day 28 vaccine) shall be administered if the following criteria **are** met on the day of vaccination:

___ 15. NOT experienced ≥ Grade 3 GVHD within past 7 days

___ 16. Disease has NOT relapsed since HCT

___ 17. Successful primary engraftment WITHOUT secondary graft failure

___ 18. NO ongoing post-HCT ≥ Grade 3 non-hem AE's, with the exception of grade 3 glucose intolerance, cholesterol, triglyceride, and hyperglycemia

___ 19. Negative for CMV viraemia: CMV qPCR <500 gc/mL from samples collected and resulted within the past 7 days

___ 20. Negative for CMV end organ disease (biopsy proven) post-HCT

___ 21. All prednisone (or equivalent) doses within the past 7 days were ≤ 1 mg/kg/day*

___ 22. NOT received any prohibited medications (Section 5.6)

___ 23. Negative pregnancy test result for females of child bearing potential

The DCC should be promptly notified by the study team of registered participants who fail to meet Day-28 Post-HCT vaccine administration criteria.

6.2 Day 56-Post-HCT Vaccine Administration Criteria

In instances where the criteria are not clear cut, the treating clinician and Site PIs will make a determination. This consultation should be documented.

Day 56 vaccine shall be administered if the following criteria **are** met:

___ 24. NOT experienced Grade 4 GVHD since Day-28 vaccination

___ 25. NO Grade 3 GVHD within the past 7 days

___ 26. Disease has NOT relapsed since HCT

___ 27. NO secondary graft failure

___ 28. NO ongoing post-HCT ≥ Grade 3 non-hem AE's, with the exception of grade 3 glucose intolerance, cholesterol, triglyceride, and hyperglycemia

___ 29. All prednisone doses (or equivalent) within the past 7 days were ≤ 1.5 mg/kg/day*

___ 30. NOT received any prohibited medications (Section 5.6)

___ 31. Negative pregnancy test result for females of child bearing potential

**Day 28 and day 56 vaccine administration criteria allow different doses of prednisone (or equivalent)*

7.0 DATA AND SAFETY MONITORING

This study will have an external, independent DMC for review of protocol events and progress, with reporting of recommendations to the COH IRB.

7.1 Definition of Risk Level

This is a Risk Level 4 study, as defined in the City of Hope Data and Safety Monitoring Plan because the trial involves a COH held IND.

7.2 Monitoring and Personnel Responsible (PMT)

The PMT will consist of the study PI, site principle investigators, collaborating investigators, Biostatistician and CRA/protocol nurse. They will be responsible for monitoring the data and safety of this study, including implementing stopping rules for safety and efficacy. In addition, monitoring of research subjects following treatment will be conducted by the Clinical Trials Office and/ or designee. Study data and safety information from all participating sites will be collected at COH for consideration by the Independent DMC prior to DMC meetings (Meeting Schedule see Section 7.6).

7.3 AE and UP Definitions

7.3.1 Adverse event (AE)

An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

7.3.2 Unexpected Adverse Event [21 CFR 312.32 (a)]

An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

7.3.3 Expected Adverse Event

Any event that does not meet the criteria for an unexpected event OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

7.3.4 Serious Adverse Event (SAE) [Modified from 21 CFR 312.32]

Serious adverse events will be reported to the sponsor (COH) within 1 business day of study staff becoming aware of them. A serious adverse event is defined as any expected or unexpected adverse event up to Day 100 that results in any of the following outcomes:

- Death
- Life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect

- Secondary Malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasisas or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

7.3.5 Unanticipated problems Involving Risk to Subjects or Others

An unanticipated problem is any incident, experience or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

7.3.6 AE Description and Grade

The descriptions and grading scales found in the most recent version of Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized to characterize AEs, a copy of which can be found at http://evs.nci.nih.gov/ftp1/CTCAE/About.html. AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.

7.3.7 AE Attribution

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

7.4 Routine Reporting of Adverse Events by Site Investigators

Adverse events of all grades will be reported into the eCRFs by the study CRA. Adverse events recorded in the case report forms include:

- All events, regardless of grade considered possibly, probably or definitely related to study agent
- All grade 3/4/5 events regardless of attribution
- All serious adverse events

Information should include: participant ID, date of the event, whether the event meets the definition of serious, whether the event is an unanticipated problem, grade of event, attribution of event, whether the event is a known expected toxicity to study agent. Provide all possible causality to the event (e.g. subject's disease, medical history, comorbidity(ies))

All adverse events must be followed until the event is resolved, stabilized, or determined to be irreversible by the participating investigator; for ongoing adverse events that are unrelated to study agent, the follow-up period may end at the 30-days post study-drug assessment. The Coordinating Center should be consulted prior to ending the follow-up of events that have stabilized.

7.5 Expedited Reporting of Unanticipated Problems (UPs) by Site Investigators

Each adverse event will be assessed to determine if it meets the criteria for expedited adverse event reporting. UPs occurring at COH will be reported using the iRIS AE/UP reporting form according to the COH reporting timeline.

Non COH sites will complete the UP/SAE Coversheet (found in Appendix E) and email a scanned copy to the COH Data Coordinating Center with the subject title "CMV SAE" to DCC@coh.org as soon as possible (electronic signature on the document is acceptable). In addition, all UPs must be reported to the local IRB and copies of the IRB submission and response provided to the COH DCC. All UP/SAE reports will be forwarded immediately to study Principal Investigator (rnakamura@coh.org).

If an email receipt from Coordinating Center personnel is not received within one working day, external site personnel should call 626-256-4673 x 63968 and/or email DCC@COH.org.

All UPs reported at COH and external sites will be compiled and submitted for review by the Independent DMC.

7.5.1 Rationale for Expedited Adverse Event Reporting to Local IRB

The criteria defining expedited reporting is derived from FDA and COH reporting criteria. The following should be reported to the local IRB and Study PI within 24 hours of being aware that the event met the expedited reporting criteria:

- All unanticipated problems
- All serious adverse events regardless of relationship to study agent, study procedure, underlying disease or concomitant treatment from the first vaccine administration till 30 days after the vaccine dose.
- All serious adverse events that are considered possibly, probably, or definitely related to the study agent observed 30 days after the last dose of vaccine

Note: follow-up reports must be submitted for all events that require expedited reporting when the status of the event changes and until the resolution or stabilization of the event.

7.5.2 Reporting of Unanticipated Problems to the FDA

SAEs meeting the requirements for expedited reporting to the FDA, as defined in 21 CFR 312.32, will be reported by COH as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting which can found at:

http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

The COH PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the following:

- any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)];
- any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

7.6 Independent Data Monitoring Committee (DMC)

Aggregate data (see Tables 1-5 of the DMC Charter) will be compiled by the Study Statistician. The COH DCC will report this study data to the independent Data Monitoring Committee. The Independent DMC will comprise three members with appropriate scientific and medical expertise to monitor the study progress (see Section 9.1 DMC Charter for Committee members). The DMC charter has been written by COH and agreed by all DMC members prior to the initial DMC meeting. A DMC chairperson will be appointed who will be responsible for conducting the meeting and summarizing the minutes of the closed portions of the meeting. All meetings will be held by teleconference, approximately a month after the following occurs:

- approx 24 patients reach day 100
- approx 48 patients reach day 100
- approx 72 patients reach day 100
- At study conclusion (optional final DMC meeting)

After any NRM within 100 days, grade 3-4 aGVHD, grade 3-4 reaction with an attribution of ‘probably’ or ‘definitely’ related to vaccination (within 2 weeks of a vaccine administration), or any other concerning reaction as deemed by the PMT, the DMC chair will be provided information and will determine if a DMC review is required. This information will be provided to the DMC chair as soon as practically possible after PMT review.

NOTE. Modifications to this schedule may be required due to study progress/findings.

7.7 Toxicities to CMV-MVA Triplex

7.7.1 Expected (known) toxicities to CMV-MVA Triplex

Expected (known) to be associated with CMV-MVA Triplex (in agreement with IB) in healthy volunteers and HCT recipients, with the highest grade indicated:

Cutaneous reaction (grade 3), Myalgia (grade 1-2), Malaise (grade 1-2), Headache (grade 1-2)

7.7.2 Anticipated toxicities to CMV-MVA Triplex

Anticipated toxicities that have not yet been seen from the agent but are foreseeable based on other similar agents include bruising at the site of injection and transient hypotension.

All unsolicited AE data will be collected up to one month post vaccination. All SAE data will be collected throughout the duration of the study, for up to one year.

7.8 Toxicities to the placebo

7.8.1 Expected (known) toxicities to placebo

Since the placebo formulation has yet to be administered to participants, there are no expected (known) toxicities associated with administering the placebo.

7.8.2 Anticipated toxicities to placebo

Anticipated toxicities that have not yet been seen from the placebo but are foreseeable based on other similar agents include bruising at the site of injection and transient hypotension.

8.0 AGENT INFORMATION

8.1 CMV-MVA Triplex and placebo –information applicable to vaccine components

CMV-MVA Triplex injection will be prepared by the investigational pharmacy from the following component provided by the COH CBG:

- cGMP CMV-MVA Triplex vaccine vials containing $5.1x10^8$ pfu/mL (Lot#0786-181-0001-1) OR $9.1x10^8$ pfu/mL (Lot#0786-181-0002-1).

  COH CBG has filled vials with cGMP CMV-MVA Triplex vaccine without lyophilization in the formulation buffer of PBS containing 7.5% lactose. Vials contain approximately 1 mL.

The placebo will be prepared by the investigational pharmacy from the following components provided by the COH CBG:

- PBS containing 7.5% lactose.

For all of the above listed components, the following elements apply:

8.1.1 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of each of the agents using the NCI Drug Accountability Record or another comparable drug accountability form.

In addition, documentation of the vaccine preparation (placebo or CMV-MVA Triplex) is required.

8.1.2 Handling

Qualified personnel, familiar with aseptic technique and procedures that ensure the quality of the agent and minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and, when applicable, safe disposal of the agent.

8.1.3 Ordering

The COH IDS pharmacy will order all agents from COH CBG in coordination with and as approved by Dr. Diamond (ddiamond@coh.org) and Dr. Nakamura (rnakamura@coh.org).

The MDA pharmacy and Dana-Farber pharmacy will order all agents from the COH IDS pharmacy.

8.1.4 Destruction and Return

Vials used in the preparation of vaccine for administration and any residual agents there in may be disposed of by the research pharmacy according to approved institutional standard of practice or policy. Unused agent will either be returned to the sponsor or disposed of according to approved institutional standard of practice. No unused agent will be disposed of without prior written approval by Dr. Don Diamond (ddiamond@coh.org).

8.2 CMV-MVA Triplex

8.2.1 Description

The CMV-MVA Triplex vaccine is a multiple-antigen recombinant MVA with genes encoding 3 major CMV proteins: UL83 (pp65), UL123 (IE1), and UL122 (IE2). The CMV-MVA Triplex vaccine was manufactured at COH CBG, in a California Food and Drug Branch (CFDB) licensed manufacturing facility which operates under the principles of cGMP for the manufacture of phase I/II biologics. The release testing of the vialed CMV-MVA Triplex vaccine to be used in the current Phase II trial was performed by BioReliance Corporation (Rockville, MA) and Wuxi-Apptec (Marietta, GA), in compliance with the requirements of the FDA Good Laboratory Practice Regulations (21 CFR 58). The vaccine passed all applicable release tests specified by the FDA. The toxicology testing of the cGMP CMV-MVA Triplex vaccine was performed at the Southern Research Institute (Birmingham, AL; Study # 13928.01.01). The IND is held by COH (BB-IND #15792).

8.2.2 Toxicology

Most common AEs included mild to moderate cutaneous reactions at the injection site. The duration of related Grade 1 and 2 AEs ranged from 1-2 days. One cutaneous grade 3 AEs (skin induration) was reported which was resolved in 2 days.

8.2.3 Supplier

CMV-MVA Triplex vaccine is being produced, formulated and provided by COH CBG. COH IDS pharmacy or COH CBG will supply MDA and Dana-Farber with CMV-MVA Triplex vaccine for this study.

CMV-MVA Triplex vaccine is supplied frozen at $5.1x10^8$ pfu/mL or $9.1x10^8$ pfu/mL in the formulation buffer of PBS containing 7.5% lactose. COH CBG has filled vials with cGMP CMV-MVA Triplex vaccine without lyophilization as a sterile, preservative-free, solution packaged in 2 mL polypropylene cryogenic vials with silicone washer seals. Each vial contains approximately 1mL (0.9 - 1.1mL).

8.2.4 Storage and stability

CMV-MVA Triplex is to be stored in a monitored freezer between -60 to -90 °C. Stability analyses of CMV-MVA Triplex are performed every 6 months.

8.2.5 Handling, Ordering, Accountability, Destruction and Return

See section 8.1.

8.3 PBS containing 7.5% lactose solution for the placebo

8.3.1 Description

PBS containing 7.5% lactose is a sterile, nonpyrogenic solution which is the diluent used in the formulation of CMV-MVA Triplex. This solution will be used for the placebo injection.

8.3.2 Toxicology

There are no known warnings. Lactose is a reducing sugar commonly used in multiple drugs as an excipient or bulking agent. It is a natural disaccharide consisting of galactose and glucose [190]. It can be administered by different routes including IM, as in SOLU-MEDROL® (Pfizer, NY) the anti-inflammatory glucocorticoid, often prescribed to HCT patients as in immunosuppressive agent. There are no restrictions for diabetes and lactose intolerant patients to take lactose containing medicines, since the amount of lactose delivered in drugs is minimal[190].

8.3.3 Formulation

PBS containing 7.5% lactose will be supplied in a 1.2 mL polypropylene cryovial vial with a fill volume of approximately 1 mL/vial. The solution contains no bacteriostat, antimicrobial agent or added buffer.

8.3.4 Supplier

PBS containing 7.5% lactose is produced by COH CBG. The COH IDS pharmacy or COH CBG will supply PBS containing 7.5% lactose to MDA and Dana-Farber.

8.3.5 Storage and Stability

PBS containing 7.5% lactose should be maintained between -60°C and -90°C in a temperature-monitored freezer. The release testing for PBS containing 7.5% lactose fill includes sterility, bacteriostasis and fungistasis, endotoxin, pH and particulate testing. The testing will be performed until the end of the study.

8.3.6 Handling, Availability, Ordering, Accountability, Destruction and Return

See section 8.1.

8.4 Preparation of CMV-MVA Triplex

A list of reagents, equipment and supplies needed to prepare the vaccine is located in Appendix D.

Note: the CMV-MVA Triplex vaccine must be administered within 4 hours after the CMV-MVA Triplex vaccine has thawed.

Thawing and preparing CMV-MVA Triplex injection

Before the scheduled patient administration, obtain frozen CMV-MVA Triplex vaccine vial from -70°C freezer in the Pharmacy.

- Put cooling block in hood and set to 4-8°C. Once the LCD on the cooling block reads 4-8°C, measure the temperature of the cooling block using the NIST thermometer and Enviro-Safe liquid or digital thermometer. Adjust LCD until thermometer reads 4-8°C. Only the thermometer should be relied upon for accuracy.
- Allow vaccine vial to thaw at room temperature (approximately 15-30 minutes). Record time at which vial is completely thawed. This is the start time of the vaccine dose preparation. Once thawed, place vial on the cooling block maintained at 4-8°C. If there is any delay between thaw and the subsequent steps, keep the thawed vial on the cooling block until it is vortexed and spun.

- Thoroughly wipe the exterior of the vial(s) with an alcohol swab.
- Vortex vial for 30 seconds at highest setting (this is to minimize clumping).
- Spin for 5 seconds in a microfuge at 6000 rpm (this is to maximize the extractable volume) .
- Unscrew the cap from the vaccine vial and withdraw CMV-MVA Triplex into an appropriately sized sterile syringe. If using Lot#0786-181-0001-1, withdraw the entire contents of vial (0.9-1mL of vaccine). If using Lot#0786-181-0002-1, withdraw 0.55 mL.
- Record the time that the CMV-MVA Triplex vaccine is placed in an insulated container with ice packs and is ready for transport.

Labeling of the vaccine to communicate necessary information and maintain the blind

- The prepared syringe or accompanying documents should be labeled in a manner to maintain a blind randomization status with the clinical study team and participant. After labeling appropriately, place syringe in sealable, plastic amber bag and place in container with ice packs.
- The time by which the vaccine needs to be administered – start time +4 hours – must be clearly noted in order to inform the person administering the vaccine (e.g. “Administer before xx:xx am/pm”).

8.5 Preparation of placebo

Preparation

Note: PBS containing 7.5% lactose solution for the placebo must be administered within 4 hours after the solution has been removed from the freezer.

- Put cooling block in hood and set to 4-8°C. Once the LCD on the cooling block reads 4-8°C, measure the temperature of the cooling block using the NIST thermometer and Enviro-Safe liquid or a digital thermometer. Adjust LCD until NIST thermometer reads 4-8°C. Only the thermometer should be relied upon for accuracy.
- Obtain PBS containing 7.5% lactose solution from the Pharmacy freezer. Record the time at which the PBS containing 7.5% lactose solution is removed from the freezer; this is the start time of the placebo dose preparation. Allow placebo vial to thaw at room temperature. Keep cold by placing vials on the cooling block, maintained at 4-8°C.
- Vortex the vial (containing approximately 1 mL of volume) for 30 seconds at highest setting. Centrifugation is not required.
- Withdraw 0.9-1 mL for the subject injection and cap syringe.
- After labeling appropriately, place syringe in sealable plastic amber bag and place in ice bucket. Record the time that the placebo is placed in a container with ice packs and is ready for transport.

Labeling of the vaccine to communicate necessary information and maintain the blind

- The prepared syringe or accompanying documents should be labeled in a manner to maintain a blind randomization status with the clinical study team and participant.
- The time by which the vaccine needs to be administered – start time +4 hours – must be clearly noted in order to inform the person administering the vaccine (e.g. “Administer before xx:xx am/pm”).

9.0 CORRELATIVE/SPECIAL STUDIES

9.1 Immunogenicity testing

The correlative immunogenicity studies will include monitoring levels and quality of CMV-specific $CD8^+$ T cells and highly cytotoxic memory $NKG2C^+$ NK cells, by multi color flow cytometric analyses.

9.1.1 Specimen Collection and Transport To Processing Laboratory

All participants will undergo serial blood sampling for immunogenicity testing at the schedule indicated in the Study Activity Calendar (Section 10), which, in general, includes days 28, 42, 56, 70, 84, 100, 140, 180, 270 and 365 post-HCT. A volume of approximately 30 mL of blood in heparin (green-top) tubes, will be collected, gently inverted several times to mix anti-coagulant, and kept at room temperature until transport to authorized personnel of the DET at COH or at Dana-Farber or MDA. Samples from all pre-registered participants, including Pre-HCT samples from participants who are not randomized (vaccinated), will be maintained/included for analysis. PLEASE SEE SECTION 9.2

9.1.2 Initial specimen processing, storage, and shipping

Sample processing and storage will occur at COH, MDA or Dana-Farber. PBMC will be separated from heparinized blood by standard density gradient centrifugation methods*, washed, re-suspended in 90% fetal calf serum (FCS) with 10% DMSO, aliquoted, and cryopreserved in a centrally monitored liquid nitrogen tanks at the COH DET, MDA and at the Dana-Farber Cancer Institute. Samples will be processed and stored according to institutional SOP; it is recommended to freeze 5 million cells per aliquot. Samples will be labeled with the study timepoint (e.g. Day 42), date of collection, and study protocol number (COH 14295) , participant study number and site (e.g. MDA, COH or Dana-Farber).

* Prior to the processing of the PBMCs, 3 mL of blood will be set aside for plasma extraction. This 3mLs of blood will be centrifuged at around 3,000g for 20 mins to spin down the cell fraction. After spinning, the upper layer of plasma is removed , transferred to labeled cryotubes and stored at in a suitable laboratory freezer between -20^{o}C and -70C, or liquid nitrogen. The cell fraction will be discarded. When requested by the ET Laboratory who are performing the analysis, frozen samples will be batch shipped from external sites, overnight on dry ice to COH.

9.1.3 Analytical Method

CMV-specific CD8 and CD4 T cells

Immunogenicity studies of CMV-specific T cells will be performed at the COH DET and will include immune-monitoring of levels, kinetics, poly functionality and phenotype assessment of CMV-specific T cells[15, 20, 28]. Additionally, CMV-specific T cell growth kinetics and early cell death we be monitored. The proposed Phase II blinded placebo controlled study will definitively assess the level of association between reduced CMV reactivation and CMV specific T cells.

Multicolor FACS analyses will be used to assess poly functionality and phenotype of CMV-specific T cells[15, 20, 28]. All immune-monitoring studies will be performed by flow cytometry techniques using the Gallios ^{TM} with Kaluza software (Beckman Coulter Inc, Brea CA).

We will evaluate the cellular CMV-specific response elicited by CMV-MVA Triplex by measuring the levels of the CD137 surface marker expressed on CD8 and CD4 T cells stimulated for 24 hours with either pp65, IE-1 and IE2 peptide libraries[182]. CD137 is expressed only on recently activated T cells, and its expression correlates with functional activation of T cells[183, 184]. All immunophenotyping will be conducted on freshly thawed PBMC without cultivation or stimulation in vitro. PBMC will be stained with each fluorochrome-conjugated antibody combination using standard methods with commercially fluoresceinated antibodies (BD Biosciences, San Jose, CA), as described in our published studies[140,

191]. In combination with CBC, we will be able to calculate the absolute number of pp65/IE1/IE2–specific CD8 and CD4 T cells/L.

Assessment of CMV-specific T cell growth kinetics will be performed using the carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution method for cell division tracking as previously detailed[182]. Briefly, proliferation will be analyzed using the CFSE dilution method, according to the manufacturer's procedure (Molecular Probes, Carlsbad, CA). To assess CD8 T cell proliferation, PBMC will CFSE labeled and incubated for 6 days with 1 μg/mL $pp65_{495-503}$ peptide or DMSO diluent as control, in the presence of anti-CD49b and CD28 (1 μg/mL). Cells will be then washed and co-stained with anti-CD8 (all antibodies from BD Biosciences) and FACS analyzed for CFSE fluorescence levels. Early cell death will be determined using the BD ApoAlert annexin V-FITC Apoptosis Kit (BD Biosciences) according to the manufacturer's instructions, and cells will be analyzed by FACS [192].

Natural Killer cell phenotype and function

Assessment of NK phenotype and function (cytotoxicity and cytokine production) will be performed at UMN. These studies will determine whether vaccination of allogeneic HCT-R^+ induces adaptive NK cell population changes, and increase in the highly cytotoxic memory $NKG2C^+$ NK cells, linked to CMV reactivation, critical for CMV adaptive immune response and potentially linked to relapse reduction[24-27]. Since in the pilot Phase Ib appeared to reduce reactivation, cGVHD and relapse we will assess whether vaccinated patients have increased levels of this potent subset of NK cells[25-27]. The NK analyses will be performed at a single cell level by using a 9-color flow approach[25].

GVHD Biomarkers

In the phase Ib trial of CMVPepVax in HCT recipients (COH IRB Clinical Protocol No. 12022), vaccine administration was associated with reduced risk of chronic GVHD while there was no increase in acute GVHD. To further investigate potential immunologic impacts of CMV-MVA Triplex on GVHD, the following GVHD biomarkers will be evaluated at the COH DET:

- interleukin-2 receptor α (IL2Ra), tumor necrosis factor receptor 1 (TNFR1), hepatocyte growth factor (HGF), interleukin 6 (IL6) and interleukin-8 (IL8) for systemic GVHD[171, 172]
- elafin for skin GVHD[173]
- regenerating islet-derived 3α (REG3α) for gastrointestinal GVHD[174]
- suppression of tumorigenicity 2 (ST2) for steroid-refractoriness [175]
- CXCL9 and B cell–activating factor (BAFF) for chronic GVHD[176, 177]

The levels of these GVHD biomarkers will be compared between the vaccine group and placebo group, as well as between GVHD+ and – groups.

9.2 MVA vector persistence

As recommended by FDA, persistence of the CMV-MVA Triplex vaccine in the blood of vaccinated patients will monitored during the 12 month observation period after receipt of both doses of vaccine. At day 28, 56, 100, 180 and 365 one additional 3 mL blood sample with anti-coagulant will be collected for this evaluation. The method in use is detailed in paragraph 2.4.2.1. Samples collected at external trial sites will be frozen as whole blood, or cell fractions prior to batch shipping to COH for analysis. If samples are collected in non glass vacutainer tubes, they can be frozen in the collection tube with no further processing. Whole blood or cell fractions should be frozen and stored in a suitable laboratory freezer between -20°C and -80°C until shipping to COH. DNA will be extracted from the white cell fraction of the blood and qPCR analysis performed. The analysis will be performed in 1 single batch at end of the study after un-blinding procedures have been completed.

10.0 STUDY CALENDAR

Table 2: Study Activity Calendar

	Pre-HCT				Vaccination and Bi-Weekly Visits [b]						Post-100 Day Follow-Up [c]			
Study day [a]	**(-60 - 0)		28[d]	42	47	56[d]	70[e]	77	84	100	140	180	270	365
Informed Consent [f]	X													
Medical history and demographics [g]	X													
Concurrent medications [h]	X [i]		X [j]	X [j]		X [j]	X [j]		X [j]	X [j]	X [k]	X [k]	X [k]	X [k]
Physical exam and vital signs [l]	X		X			X								
KPS performance status [m]	X		X			X								
Adverse event (CTCAE) assessment [n]			X	X		X	X		X	X				
Engraftment status [o]		HCT Day 0	X	X		X	X		X	X	X	X	X	X
Disease relapse [p]			X	X		X	X		X	X	X	X	X	X
GVHD assessment and grading [q]			X [r]	X		X [s]	X		X	X	X	X	X	X
CMV disease [t]			X [u]			X [u]								X [v]
CMV qPCR [w]			X	X		X	X		X	X	----------X[x]----------			
HIV, HCV, CMV, active HBV [y]	X													
Pregnancy test [z]	X		X			X								
Chemistry/metabolic panel [aa]	X		X			X								
CBC with differential [bb]			X	X		X	X		X	X	X	X	X	X
Research blood sample [cc]			X*	X		X*	X		X	X*	X	X*	X	X*
Criteria review	X [dd]		X [ee]			X [ff]								
Registration/verify with DCC	X [gg]													
Vaccine administration [hh]			X[hh]			X[hh]								
EKG					X[ii]			X[ii]						

** Criteria review, screening, consent and Registration with DCC permitted Day -60 to Day 0

a. Study day is defined relative to the day of HCT which is defined as Day 0.
b. Window for post-HCT Day 28 and Bi-Weekly Assessment procedures is the assigned day +/- 5 days.
c. Windows for the 'Post-100 Day Follow-Up' visits is the assigned day +/- 15 days.
d. All assessments to be performed and reviewed on the day of and prior to vaccine administration, except for CMV qPCR for which the result may remain pending.
e. On Day 70 post-HCT, 14 days after the second vaccination, administration of other vaccines is no longer prohibited (Section 5.6).

f. Informed consent process to be fully documented: e.g. prospective participant had sufficient time for deliberation, all questions were answered, treatment options provided by MD, full study reviewed including risks, and a copy of signed consent given to participant. Informed consent must occur prior to any research only (non-SOC) screening procedures.

g. Medical history and demographics– to include any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.

h. Concurrent medications, supportive care, blood products, or radiation therapy taken or administered during the trial will be documented in the subject's medical record using institutional documentation guidelines.

i. Concurrent medications pertaining to eligibility criteria will be reviewed.

j. Concurrent medication data collected in CRFs pertains to anti-viral medications (including start and stop date), immunosuppressive agents, daily prednisone dose for 7 days prior to vaccine administration, and prohibited medications (Section 5.6).

k. Concurrent medication data collected after 100 days is limited to anti-viral medications (including start and stop date).

l. Physical exam to include skin assessment. Vital signs: Weight, heart rate, blood pressure, respiration rate, temp. Height required only at baseline.

m. KPS scale is found in Appendix C.

n. Adverse events (AEs) will be assessed documented in the source documents and the case report forms in the defined study visits and at study of care visits. All grade 1 and 2 AEs that occur within 2 weeks from each vaccination will be reported. All grade 3/4/5 AEs will be reported from Day 28 to Day 100. All serious adverse events as defined in Section 7.3.4 will be reported up to Day 100. All AEs considered possibly, probably or definitely related to study agent will be reported till the end of the study period (Day 365). See Section 7.0 for AE reporting.

o. Engraftment status should be documented at each study visit, and if engraftment failure occurs the date of engraftment failure should be noted (see Section 5.3.3 for definitions).

p. See Section 5.3.4 for definition of disease relapse. Disease relapse will be assessed according to and per the timing of institutional SOC practice for the participant's specific hematologic malignancy. **Note:** participants who undergo disease relapse will cease all future study visits/procedures and will be followed only for survival through Day 365.

q. Acute and chronic GVHD grading scales are found in Appendix A and B, respectively. The final grading may occur after a clinic visit has ended, using all diagnostic information available to determine the GVHD grade at the time of the visit. In the study CRF, the grade at the time of the study visit will be recorded, and the highest grade and date of onset during the interim period between visits (assessed at SOC visits), if higher than the grade at the visit.

r. For the Day 28 visit, assessment and grading of GVHD for the 7 days prior to the visit must be determined and documented on the day of the visit to assess/support vaccine administration criteria. The determination may be revised as more information is available, but will be documented in the medical record and in the CRF as a revised GVHD assessment and grading following new information obtained after vaccine administration.

s. For the Day 56 visit, assessment and grading of GVHD between the initial vaccination and Day 56 must be determined and documented on the day of the visit to assess/support vaccine administration criteria. The determination may be revised as more information is available, but will be documented in the medical record and in the CRF as a revised GVHD assessment and grading following new information obtained after vaccine administration.

t. Clinically confirmed CMV disease will be captured in the case report forms. Presentations or suspected presentations of CMV disease in the absence of qPCR >500 gc/mL will be evaluated by the treating investigator in conjunction with the blinded PMT before a determination is made.

u. Participants with suspected CMV disease on the day of planned vaccination must have testing to confirm the presence or absence of CMV disease prior to determining for the CMV disease vaccination administration criterion.

v. For the Day-365 visit, the treating investigator will investigate and document whether CMV disease occurred Day 100 onward, and so note the findings accordingly. The case report forms will be updated to record any positive results not yet documented.

w. CMV qPCR will be performed per institutional SOC (usually twice weekly), and at minimum weekly between days 21 and day 100 post-HCT. CMV results will be collected in the case report forms.

x. Post Day 100, CMV qPCR to be performed only if clinically indicated or per institutional SOC. All CMV qPCR results will be collected in the case file.

y. CMV serostatus, HIV antibody, Hepatits C antibody, Hepatitis B surface antigen test must be performed if results are not available within 2 months of registration. If CMV seropositive status not already documented, CMV serotesting may be performed for eligible review.

z. Serum or urine pregnancy test to be performed for women of child bearing potential.

aa. Chemistry/metabolic panel to include: glucose, BUN, creatinine, uric acid, total proteins, albumin, calcium, phosphorous, sodium, potassium, chlorine, total CO_2, total bilirubin, alkaline phosphatase, ALT, AST, LDH, total cholesterol.

bb. CBC with differential is to be taken at the same time as the research blood sample.

cc. Research blood samples are to be collected at the same time as the CBC with differential. A volume of approximately 30 mL of Blood will be collected in heparin (green-top) tubes, gently inverted several times to mix anti-coagulant, and then kept at room temperature until transport to authorized personnel of the COH DET, MDA or Dana-Farber Research laboratory. Approximately 27 ml and 3 ml are allocated for PBMC and plasma isolation respectively. * indicates that at Day 28, 56, 100, 180, 365 one additional tube with approx 3 mL of blood will be collected for the evaluation of the persistence of the MVA vector (see Section 9.2). In addition, in the case of a detectable CMV reactivation, external study sites will collect an extra tube (5mls, EDTA) at each research blood time point until day 100, for CMV harmonization measurements.

dd. Eligibility criteria for enrollment in the clinical trial are found in Section 3.0.

ee. Day-28 Vaccine Administration Criteria are found in Section 6.1. Participants failing to meet Day-28 vaccine criteria will complete the study at this time and be replaced and the study team should promptly inform the DCC.

ff. Day-56 Vaccine Administration Criteria are found in Section 6.2. Participants failing to meet Day-56 vaccine criteria will continue with remaining study procedures; participants with disease relapse will continue for survival follow-up only.

gg. The study team will submit copies of source documentation, the eligibility criteria list, signed consent, and registration cover sheet to the DCC who will proceed to confirm study eligibility and register the participant. See Section 4.2 for details.

hh. Vaccine administration is detailed in Section 5.0.

ii. Monitoring for cardiac AEs by EKG can be performed +/- 10 days of the assigned visit.

11.0 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

The primary aims of this trial are to estimate the efficacy of CMV-MVA Triplex in reducing the frequency of CMV events, and to further evaluate the safety and tolerability in HCT recipients. Secondary endpoints will include additional safety, clinical and immunological parameters. In detail:

Primary endpoints:

1. CMV events encompassing any CMV reactivation [≥500 CMV genome copies (gc)/mL], low-level reactivation prompting antiviral therapy, or CMV disease prior to day 100 post-HCT.
2. Key safety endpoints: non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and grade 3-4 AEs (CTCAE v.4.0) probably or definitely related to the vaccination within 2 weeks from each vaccination.

Secondary endpoints:

1) CMV-related events: time-to viremia (defined as number of days from transplantation to the date of >500 CMV gc/mL), duration of viremia, incidence of late CMV viremia (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia of ≥1500 CMV gc/mL), cumulative number of CMV specific antiviral treatment days.
2) Transplant-related events: time to engraftment, incidence of aGVHD, chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, infections.
3) Immunological function: levels and kinetics of CMV-specific T cell immunity, combined with immunophenotyping[19-21], and functional studies[22, 23]. NK phenotype and function (cytotoxicity and cytokine production).

12.0 STATISTICAL CONSIDERATIONS

12.1 Study Design

This is a randomized, blinded and placebo controlled Phase II trial conducted at three centers, COH, MDA and Dana-Farber with data coordination at COH. The primary aims of the trial are to assess the ability of CMV-MVA Triplex to protect CMV-positive HCT recipients (HCT R+) from CMV reactivation events, as defined in Section 11, and to evaluate safety. A secondary aim is to estimate the effect of CMV-MVA Triplex on levels of CMV cellular immunity in HCT-R+[161-163] and to describe the association of CMV cellular immunity with CMV reactivation events. Additional secondary aims are to evaluate the effect of CMV-MVA Triplex on other transplant-related outcomes and on the duration and treatment of viremia. The study includes rules for periodic safety monitoring.

12.2 Randomization

The trial has a target of 102 randomized HCT recipients. Eligible HCT-R+ will be consented and enrolled pre-HCT, and registered through COH DCC. A computer-generated randomization, stratified by donor CMV serostatus and center, will assign registered participants to the CMV-MVA Triplex or placebo arm; the treatment assignment will be provided to site pharmacists, who are unmasked to treatment-group allocation, in advance of planned vaccination. Registered participants will be followed for the course of transplant and be assessed for the eligibility for 'Day-28 post-HCT' vaccination.

Participants who meet the initial vaccine administration criteria will receive the initial injection (vaccine or placebo); participants failing to meet the criteria will be replaced. Only participants who receive an injection (CMV-MVA Triplex or placebo) will be considered "randomized", as there is no opportunity for a drop-out bias prior to injection in this double-masked study. All participants who receive a first injection will be included in the primary analysis, regardless of receipt of the second injection, consistent with the intention-to-treat principle. Information regarding registered participants who do not receive the planned injection will be entered into the computer-generated randomization log to inform subsequent treatment assignments (their assignments will be replaced at randomly designated slots in the randomization log). Participants who do not meet the criteria to receive the first injection (CMV-MVA Triplex or placebo) will leave the study, without further treatment or follow-up, but their data collected up to study departure will be included in the trial dataset as non-randomized subjects, and these data will be incorporated into estimates of CMV event rates post-HCT based on the intention to treat principle.

12.3 Sample Size Accrual Rate

A 40% rate of viremia (CMV reactivation) is expected among unvaccinated, eligible HCT recipients, with some possibility of a rate as low as 30%. Based on the aim to detect either a vaccine-effect resulting in a drop from 40% to 15% or a drop from 30% to 10%, we plan to enroll and inject 51 subjects in each group. The sample size will provide at least 90% power under either scenario, at a one-sided 0.10 level of significance (appropriate to a phase II trial, and conservatively estimated based on binomial incidence). The sample will also allow at least 83% power to achieve a 0.05 level of significance. Allowing for 10%-20% of subjects becoming ineligible before vaccination, we expect to enroll approximately 114-130 subjects in 2 years, to vaccinate 102-104, and observe between 20 and 28 reactivation events. The 102-104 HCT-R+ will be randomized, at or near day 28, in an approximate 1:1 ratio, to either the CMV-MVA Triplex vaccine arm, or to the placebo arm. Over the past five years, COH, MDA and Dana-Farber combined have performed on-average >300 adult allogeneic HCT procedures annually. We anticipate about 150 CMV seropositives to be eligible annually. Accrual should be completed in <4 years from the start date of the trial, and we anticipate 1 year of follow up and data analysis.

12.4 Data Analysis Plan

CMV Events

For the primary efficacy aim, each randomized study subject will be followed according to the study calendar (see Study Calendar, section 10.0) for the occurrence of CMV reactivation events, which are defined in section 11 in terms of either viremia, low-level viremia treated with antivirals, or CMV disease. Both initial and recurrent events will be recorded, with patients considered at risk for recurrent events after completion of a full planned course of anti-viral therapy. If PCR results from blood drawn on the day of first injection should indicate reactivation, the subject will be replaced and excluded from the primary comparison, but the subject will, in all other respects, continue on the study with all planned treatments and data collections. Vaccine and placebo groups will be compared with regard to the hazard of CMV events, using the Anderson-Gill approach to repeated events, as implemented in the R survival package, as we have previously published. Power calculations are conservatively based only on binomial incident event rates.

Safety

The primary safety aim will involve both safety monitoring of the vaccine arm, described below in section 12.5, and comparison of arms at the end of the study. Non-relapse mortality at 100 days will be summarized by arm as percentages, with exact binomial confidence bounds, and compared across arms using Fishers exact test. The incidence of severe acute GVHD will be summarized by Kaplan-Meier curves, and compared using the log-rank test. Any grade 3-4 SAE within two weeks of injection that are at least possibly attributed to the injection by the (masked) treating physician, will be listed, with incidence compared by Fisher's exact test.

Cellular immunity

The data analysis for the secondary aim of estimating the effect of vaccination on cellular immunity will necessarily be more exploratory in nature. The longitudinal CMV-specific cellular assay data will be modeled on a logarithmic scale, using a generalized estimating equation approach to accommodate the stochastic dependence through time. This produces an estimated multiplicative effect of vaccination, qualified by a valid estimate of variability.

Viremia duration and treatment

The total days on antivirals for CMV reactivation (induction, maintenance, and total) will be assessed for each individual, with arms compared using Wilcoson's rank-sum test. Other characterizations of the duration of viremia and response to therapy will be descriptive in nature.

Transplant-related events

The cumulative incidence of acute GVHD, chronic GVHD, relapse-free survival, and infections will be estimated using Kaplan-Meier or Fine-Gray estimators, as appropriate, and compared using the log-rank test, or the corresponding subdistribution test due to Gray. Computing will be dose using the survival and cmprsk R packages.

12.5 Safety Monitoring

Clinical data will be monitored as they accumulate, and vaccination will be suspended for safety review if there is evidence of serious treatment-related AEs. Specifically:

(1) 100 days non-relapse mortality (NRM) will be monitored as the 12th, 24th and 36th subject on the vaccine arm reaches the 100 day evaluation point. Operationally, the CRA will notify the monitoring statistician as cohorts of 24 patients (12 vaccinated) near the 100 day mark. If NRM frequencies exceed 4, 6, or 8 patients, at the designated 100 day evaluation point, then the trial will be suspended for DSMB safety review. These numbers were selected to limit the overall false-alarm probability for this endpoint to less than 0.02 when there is no additional risk due to immunization.

(2) Severe acute GVHD (aGVHD, grade 3-4) will be monitored as the 12^{th}, 24^{th}, and 36^{th} subject on the vaccine arm reaches the 100 day evaluation point. The trial will be interrupted if 6 or more of the first 12 recipients, or 9 of 24, or 11 of 36, experience Grade 3-4 aGVHD. This would be a significant elevation from the COH/MDA/Dana-Farber historical benchmark of 15% of allogeneic HCT recipients with matched sibling donors [30]. These rules are determined to limit the overall aGVHD false alarm probability to 0.02 if vaccination does not increase risk[30].

(3) Serious AEs (SAE, grade 3-4) probably or possibly related to the vaccine (within 2 weeks from each vaccination) will be individually reviewed by the protocol monitoring team, and reported to the DSMC.

Interim monitoring involves judging the vaccine arm against fixed benchmarks, to maximize power early in the study. Comparison of treatment groups with respect to safety endpoints will occur at the end of the trial, and will use standard time-to-event statistical methods.

13.0 DATA HANDLING, DATA MANAGEMENT, RECORD KEEPING

13.1 Source Documents

Source documents are original documents, data, and records (e.g., medical records, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. The Site Investigator or their designee will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each patient enrolled in this clinical trial. Source documents must be adequate to reconstruct all data transcribed onto the case report forms.

13.2 Data Capture Methods and Management

Data for this trial will be collected using Medidata RAVE, City of Hope's electronic capture system. Medidata RAVE is a web based, password protected system that is fully compliant with global regulatory requirements, including 21CRF Part 11 compliant.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF). A system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

The Data Coordinating Center will run monthly data expectation reports that will list any outstanding and overdue data. The Data Coordinating Center will send via email to the participating site a report monthly on any missing and/or overdue data forms. The participating site will be required to complete the missing and/or overdue data forms within 1 week of receipt of the report.

Query reports will be generated on a monthly basis by the Data Coordinating Center. The Data Coordinating Center will send via email to the participating site a report monthly on any outstanding queries.

The participating site staff (whether Principal Investigator or the staff collecting data at site) are required to take an eLearning Module within Medidata RAVE in order to obtain full access. The participating site staff will receive training via teleconference by COH DCC staff to review eCRFs that are specific to this protocol. Continuous training will be offered to participating sites if any amendments affect changes to the eCRFs during the course of the trial. The eCRFs within Medidata RAVE for this trial will have detailed instructions in the form of Help Text that provide instructions for completing each required field on each form.

13.3 Case Report Forms/Data Submission Schedule

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. All case report forms must be completed by designated study personnel. The completed case report forms must be reviewed, signed and dated by the Site Investigator or designee in a timely fashion.

All data will be collected using electronic data collection system described in Section 13.2, and will be submitted according to the timelines indicated in Table 2 (below).

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 14 calendar days of treatment administration
Adverse Event Report Forms	Within 14 calendar days of the study visit
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms (concomitant medications, chemistry, hematology etc.)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of completing treatment or being taken off study for any reason

Table 2: Data Submission Schedule

13.4 Regulatory Records

The Investigator will maintain records, including updating records in accordance with Good Clinical Practice guidelines and FDA regulations. Additional information regarding required documents is provided in the DCC Operations Manual, a supplement to this protocol.

14.0 ADHERENCE TO THE PROTOCOL

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. Protocol deviations may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

All deviations from the protocol must be documented in study subject source documents and promptly reported to the Study PI. Protocol deviations will be submitted according to study site procedures to the local IRB (e.g. IRIS at COH), but programmatically exempted from local DMC review. A deviation report, which will include protocol deviations, will be prepared every 3 months for consideration by the COH PMT and Independent DMC.

14.1.1 Emergency Modifications

Investigators may implement a deviation from the protocol to eliminate an immediate hazard(s) for the protection, safety, and well-being of the study patient to trial subjects without prior IRB or Sponsor approval.

For any such emergency modification implemented,

- the local IRB must be notified according to local institutional policies.
- the Study Principal Investigator must be notified as soon as practicable (within 24 hours) via email to rnakamura@coh.org and dcc@coh.org. This email should provide input on the following:
  - Description of the event
  - Impact on participant safety or the safety to others
  - Impact on the study design

14.1.2 Planned Non-Emergency Deviations

All non-emergency planned deviations from the protocol must have **prior** approval by the Study Principal Investigator, the Site Principal Investigator and the local IRB, and if applicable the COH IRB.

Unplanned Deviations – Deviations Discovered After They Have Occurred

For deviations to the protocol discovered after they have occurred,

- the local IRB must be notified according to local institutional policies.
- the Study Principal Investigator must be notified as soon as practicable (within 24 hours of awareness of event) via email to rnakamura@coh.org and dcc@coh.org. This email should provide input on the following:
  - Description of the event
  - Impact on participant safety or the safety to others
  - Impact on the study design
  - A corrective and preventative action plan

A list of deviations from all participating sites will be submitted along with the PMT progress report to the external DMC.

15.0 STUDY OVERSIGHT, QUALITY ASSURANCE, AND DATA & SAFETY MONITORING

15.1 Site Principal Investigator

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation.

The Investigator agrees to: Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when necessary to protect the safety, rights or welfare of subjects. Personally conduct or supervise the study (or investigation). Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee). Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study. Promptly report to the IRB and the Sponsor (see Section 14) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports). Seek IRB and Sponsor (see Section 14) approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

15.2 Study Principal Investigator

The Study Principal Investigator is responsible for the conduct of the clinical trial, including overseeing that sponsor responsibilities as defined in § 21 CFR 312. Subpart D is executed in accordance with federal regulations.

15.3 Protocol Management Team (PMT)

The Protocol Management Team (PMT) minimally consisting of the study principal investigator, site principal investigators, collaborating investigators, the research nurse, the clinical research associate/coordinator, and the study biostatistician is responsible for ongoing monitoring of the data and safety of this study, including implementation of the stopping rules for safety and efficacy.

The PMT will meet (in person or via teleconference) at least monthly to review study status. This review will include, but not be limited to, reportable AEs and UPs, and an update of the ongoing study summary that describes study progress in terms of the study schema. The meeting will be a forum to discuss study related issues including accrual, SAE/AEs experienced, study response, deviations/violations and study management issues. The appropriateness of further subject enrollment and the specific intervention for subsequent subject enrollment are addressed.

PMT reports will be prepared at COH using the PMT report form in IRIS. Since there is an Independent DMC for this study, PMT reports will be exempt from COH Committee review, but stored as 'submitted' in IRIS. PMT reports will be retrieved by the study CRA for review by the Independent DMC as necessary.

15.4 Monitoring

The Investigator/Institution will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and regulatory requirements, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by the City of Hope Office of Clinical Trials Auditing and Monitoring (OCTAM), according to the COH OCTAM SOP which is provided as a supplement to this document.

Staff from OCTAM will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the City of Hope Office of Clinical Trials Auditing and Monitoring SOP. Documentation of monitoring activities and findings will be provided to the site study teams, the site PI, study PI and Independent DMC.

15.5 Quality Assurance

The City of Hope Clinical Research Information Support will provide quality assurance as detailed in the COH DCC Operations Plan Manual.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Ethical Standard

This study will be conducted in conformance with the principles set forth in *The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research* (US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979) and the Declaration of Helsinki.

16.2 Regulatory Compliance

This study is to be conducted in compliance with the IRB approved **protocol** and according to the following considerations:

- US Code of Federal Regulations (CFR) governing clinical study conduct
    - Title 21 Part 11 – Electronic Records; Electronic Signatures
    - Title 21 Part 50 – Protection of Human Subjects
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
    - Title 21 Part 56 – Institutional Review Boards
    - Title 21 Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies
    - Title 21 Part 312 – Investigational New Drug Application
    - Title 45 Part 46 – Protection of Human Subjects
- US Federal legislation, including but not limited to
    - Health Insurance Portability and Accountability Act of 1996
    - Section 801 of the Food and Drug Administration Amendments Act
- Applicable state and local laws. For research occurring in California, this includes but is not limited to State of California Health and Safety Code, Title 17
- Applicable institutional research policies and procedures

16.3 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate IRB holding a current US Federal wide Assurance issued by and registered with the Office for Human Research Protections (OHRP). Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent document will be in the possession of the Investigator, and, for sites external to COH, the possession of the coordinating center, before the study is initiated. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

The IRB will be informed of serious unexpected or unanticipated adverse experiences occurring during the study and any new information that may adversely affect patient safety or conduct of the study.

Any amendment to the protocol document and accompanying informed consent document/template, as developed and provided by the Study PI, will require review and approval by the IRB before the changes are implemented in the study. The protocol and consent will be reviewed and approved by the COH IRB before submission to a participating site IRB.

16.4 Informed Consent

For a multi-site study, each participating institution will be provided with a model informed consent form. Each institution may revise or add information to comply with local and/or institutional requirements, but may not remove procedural or risk content from the model consent form. Furthermore, prior to submission to the IRB (initial submission and amendments), the consent and accompanying HIPAA form, if separate to the consent, must be reviewed and approved by the Data Coordinating Center.

After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative before study participation. The method of obtaining and documenting the informed consent and the contents of the consent must comply with the ICH-GCP and all applicable regulatory requirements.

Before implementing any study procedure, informed consent shall be documented by the use of a written consent form approved by the IRB/IEC and signed and dated by the patient or the patient's legally authorized representative at the time of consent. A copy of the signed informed consent will be given to the patient or patient's legally authorized representative. The original signed consent must be maintained by the Site Investigator and available for inspection sponsor designated representatives, or regulatory authority at any time.

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation.

16.5 Women, Minorities, Children, HIV-Positive Individuals (Special Populations)

16.5.1 Inclusion of Women and Minorities

The study is open anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue and randomize approximately 102 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

Women who are pregnant or plan to become pregnant are excluded from participation because CMV-MVA Triplex has unknown properties and has not been explored in a developmental study in children. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the administered vaccine, breastfeeding should be discontinued if the mother is enrolled on this study.

16.5.2 Exclusion of Pediatric Patients

Pediatric recipients (children <18 years old of age) are excluded from this study because insufficient data are available in adults to judge potential risks in children. Additionally, vaccine dosage and the blood volume established for immune-monitoring in adults cannot be applicable for both adults and children. Finally, the risk of CMV complications is inversely related to age, and the inclusion of younger children could bias the endpoint observations.

16.5.3 Exclusion of HIV Positive Individuals

Individuals who are positive for HIV are expected to have very different underlying immune functions and therefore may respond to the CMV-MVA Triplex vaccine differently from individuals negative for HIV.

16.6 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to participants.

This research will be conducted in compliance with federal and state requirements relating to protected health information (PHI), including the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). HIPAA regulations require a signed subject authorization informing the subject of the nature of the PHI to be collected, who will have access to that information and why, who will use or disclose that information, ant the rights of a research participant to revoke their authorization for use of their PHI. In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Release of research results should preserve the privacy of medical information and must be carried out in accordance with Department of Health and Human Services Standards for Privacy of Individually Identifiable Health Information, 45 CFR 164.508. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed and no identifiers will be used.

Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. Data will be entered, analyzed and stored in encrypted, password protected, secure computers that meet all HIPAA requirements. All data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number. Source documents provided to coordinating center for the purpose of auditing or monitoring will be de-identified and labeled with the study number, subject ID, and patient initials.

The investigator/institution will permit direct access to source data and documents by sponsor representatives, the FDA, and other applicable regulatory authorities. The access may consist of trial-related monitoring, including remote monitoring, audits, IRB/IEC reviews, and FDA/regulatory authority inspections. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

16.7 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study Sponsor prior to participation in this study. All City of Hope investigators will follow the City of Hope conflict of interest policy.

A financial disclosure form will be obtained from each external DMC member. The SMT will maintain a copy of the form with study DMC files. The DMC members will be required to provide annual updates of when their financial interests change during the life of the clinical study. DMC members who develop serious conflicts of interest that could impact objectivity may be removed from the DMC and will be replaced (see DMC Charter for further details).

16.8 Financial Obligations, Compensation, and Reimbursement of Participants

The investigational drug including CMV-MVA Triplex and the matched placebo will be provided free of charge by COH.

Neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

The standard of care drugs or procedures provided during the course of study participation will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. In the event of physical injury to a research participant resulting from research procedures, appropriate medical treatment will be available at City of Hope to the injured research participant. City of Hope will not provide financial compensation in the event of physical injury to a research participant. The research participant will not receive reimbursement or payment for taking part in this study.

16.9 Publication/Data Sharing

Any part of the results of the study carried out under this protocol, nor any of the information provided by City of Hope for the purposes of performing the study, will be published or passed on to any third party without the written approval of Dr. Don J. Diamond. Any investigator involved with this study is obligated to provide City of Hope with complete test results and all data derived from the study.

The preparation and submission for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the City of Hope and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

In accordance with the U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801, this trial will be registered onto ClinicalTrials.gov and results will be reported on ClinicalTrials.gov within 12 months of the estimated or actual completion date of the trial, whichever date is earlier.

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are

sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A: ACUTE GVHD STAGING

1994 Keystone Consensus Criteria

Organ Staging of Clinical Acute GVHD

Skin	Lower GI	Upper GI	Liver (Total Bilb)
0- No Rash I- Maculopapular rash, <25% of body surface II- Maculopapular rash, 25-50% of body surface III- Rash on >50% of body surface, or generalized erythroderma IV- Generalized erythroderma with bullous formation and/or desquamation	0- ≤500 mL/day or <280 mL/m^2/day I- >500 but ≤1000 mL/day or 280-555 mL/m^2/day II- >1000 but ≤1500 mL/day or 556-833 mL/m^2/day III- >1500 mL/day or 833 mL/m^2/day IV- Severe abdominal pain with or without ileus, or stool with frank blood or melena	0- No protracted nausea and vomiting I- Persistent nausea, vomiting, OR biopsy showing acute GVHD of stomach or duodenum	0- <2.0 mg/dL I - 2.0-3.0 mg/dL II- 3.1-6.0 mg/dL III- 6.1-15 mg/dL IV- >15.0 mg/dL

Overall Clinical Grading of Severity of Acute GVHD

Grade	Skin		Gut		Liver
I	Stage I-II	&	None /Stage 0	&	None /Stage 0
II	Stage III	Or	Stage I	Or	Stage I
III	Stage 0-IV	Or	Stage II-IV	Or	Stage II-III
IV	Stage IV	Or	Stage 0-IV	Or	Stage IV
If KPS is ≤ 30%, or decreased ≥ 40% from baseline KPS, the status is Grade IV					

APPENDIX B: CHRONIC GVHD GRADING

Onset of Chronic GVHD *Karnofsky/Lansky score at time of diagnosis	Progressive (acute GVHD progressed directly to chronic) Interrupted (acute GVHD resolved, then Chronic developed) De novo (acute GVHD never developed) Chronic GHVD Flare (symptoms reactivated within 30 days of drug tapering or discontinuation)
Diagnosis of Chronic GVHD based on	Histologic evidence/biopsy proven Clinical Evidence Both Unknown
Maximum Chronic GVHD	Limited-localized skin involvement and/or hepatic dysfunction due to chronic GVHD Extensive-generalized skin involvement; or, liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or involvement of eye: Schirmer's test with <5mm wetting; or, involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or, involvement of any other target organ
Overall Severity of Chronic GVHD	**Mild**-signs and symptoms of chronic GVHD do not interfere substantially with function and do not progress once appropriately treated with local therapy or standard systemic therapy (corticosteroids and/or cyclosporine or FK 506) **Moderate**-signs and symptoms of chronic GVHD interfere somewhat with function despite appropriate therapy or are progressive through first line systemic therapy (corticosteroids and/or cyclosporine or FK 506) **Severe**-signs and symptoms of chronic GVHD limit function substantially despite appropriate therapy or are progressive through second line therapy

APPENDIX C: KARNOFSKY PERFORMANCE SCALE

Karnofsky Performance Status

KPS 100 Normal; no complaints; no evidence of disease
KPS 90 Able to carry on normal activity; minor signs or symptoms of disease
KPS 80 Normal activity with effort; some sign or symptoms of disease
KPS 70 Cares for self; unable to carry on normal activity or do active work
KPS 60 Requires occasional assistance, but is able to care for most personal needs
KPS 50 Requires considerable assistance and frequent medical care
KPS 40 Disabled; requires special care and assistance
KPS 30 Severely disabled; hospitalization is indicated, although death not imminent
KPS 20 Very sick; hospitalization necessary; active support treatment is necessary
KPS 10 Moribund; fatal processes progressing rapidly
KPS 0 Dead

APPENDIX D: REAGENTS, EQUIPMENT AND SUPPLIES NECESSARY FOR VACCINE PREPARATION

Reagents

- CMV-MVA Triplex vaccine vials containing $5.1x10^8$ pfu/mL, fill volume approximately 1mL (Lot#0786-181-0001-1) OR $9.1x10^8$ pfu/mL, fill volume approximately 1 mL (Lot#0786-181-0002-1).
- PBS containing 7.5% lactose, fill volume 1mL

Equipment

- Vortex
- Test tube cooling block
- NIST thermometer (kept in the IDSA refrigerator) or digital thermometer
- microfuge

Supplies

- Sterile syringes 1mL and 3mL
- Sterile needles
- Small sealable amber plastic bag
- Alcohol swabs
- Container with Ice packs(provided by IDS with prepared dose)

APPENDIX E: SAE/UP REPORTING COVERSHEET

NOTIFICATION OF UNANTICIPATED PROBLEM/SERIOUS ADVERSE EVENT

For Use by Participating Institutions Only

THIS FORM MUST BE **EMAILED** TO DCC@COH.ORG WITHIN 24 HOURS OF KNOWLEDGE OF ONSET OF SERIOUS ADVERSE EVENT OR UNANCTICIPATED PROBLEM

COH IRB #14295- Participating Site IRB # _____

From:	Date:
Phone No.:	Email:

Reporting Investigator:	
Event:	
Participant ID:	Institution:
Date Event Met Reporting Criteria (as defined in protocol):	

Type of Report: ☐ Initial ☐ Follow-up

Toxicity Grade: ☐ G1/mild ☐ G2/moderate ☐ G3/severe ☐ G4/life threatening ☐ G5

Attribution to **Vaccine**: ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite

Historical/Known Correlation to **Vaccine**: ☐ Expected ☐ Unexpected

Meets Definition of Serious AE: ☐ Serious ☐ Non-serious

Meets Definition of Unanticipated Problem: ☐ UP ☐ Not a UP

Has the event been reported to the following institution's IRB? __ No __ Yes Date: __ __ / __ __ / __ __

Signature of Investigator:_____Date:_____

APPENDIX F: REGISTRATION COVERSHEET

COH IRB 14295: A PHASE II RANDOMIZED, PLACEBO-CONTROLLED TRIAL TO EVALUATE THE PROTECTIVE FUNCTION OF AN OPTIMIZED DOSE OF CMV-MVA TRIPLEX VACCINE IN RECIPIENTS OF AN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT

Data Coordinating Center: City of Hope
1500 Duarte Road
Duarte, CA 91010
Tel: 626-256-4673 x 3968
Email: DCC@coh.org (use #secure# in subject line)

Principle Investigator:_____

Participating Site:_____

CRA/Study Coordinator: _____ Contact Number: _____

<table>
<tr><td colspan="2">Patient's Initials: (F M L):</td><td>Institution:</td></tr>
<tr><td colspan="2">Patient's DOB:</td><td>Investigator/Treating Physician:</td></tr>
<tr><td colspan="2">Patient's Zip Code:</td><td>IRB approval valid until (date):</td></tr>
<tr><td colspan="2">Sex: _____Male _____Female</td><td>Date Informed Consent was Signed:</td></tr>
<tr><td>Race:
___ Black
___ Caucasian
___ Asian
___ American Indian
___ Native Hawaiian/Pacific Islander
___ Other _____</td><td>Ethnicity:
___ Hispanic
___ Non-Hispanic
___ Other _____</td><td>Projected Transplant Date:

Diagnosis: _____</td></tr>
</table>

CMV-MVA IN RECIPIENTS OF ALLO HSCT

DANA-FARBER CANCER INSTITUTE/HARVARD CANCER CENTER

CITY OF HOPE

REGISTRATION COVER SHEET

STUDY INFORMATION

Sponsor: NIH: NCI: Division of Cancer Prevention and Control

DFCI IRB #: 15-555

SUBJECT INFORMATION

Subject Number: _____

Subject initials: _____

Year of Birth: _____

First 3 digits of zip code: _____

Gender: _____

Race: _____

Ethnicity (circle one): Hispanic / Non-Hispanic / Unknown

Diagnosis: _____

Date of Consent: _____

Projected HSCT Day 0:_____

RESEARCH TEAM INFORMATION

Name of Consenting Investigator: _____

Name of Registering Person: _____

Contact Phone of Registering Person: _____

Investigator Signature: _____ Date:_____

Registering Person Signature: _____ Date:_____