City of Hope

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

CITY OF HOPE PROTOCOL VERSION: #19065 **Protocol Date:** 09/28/22

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Amendment 10	Protocol Dated 07/15/22	Packet 10
Amendment 11	Protocol Dated 08/25/22	Packet 11
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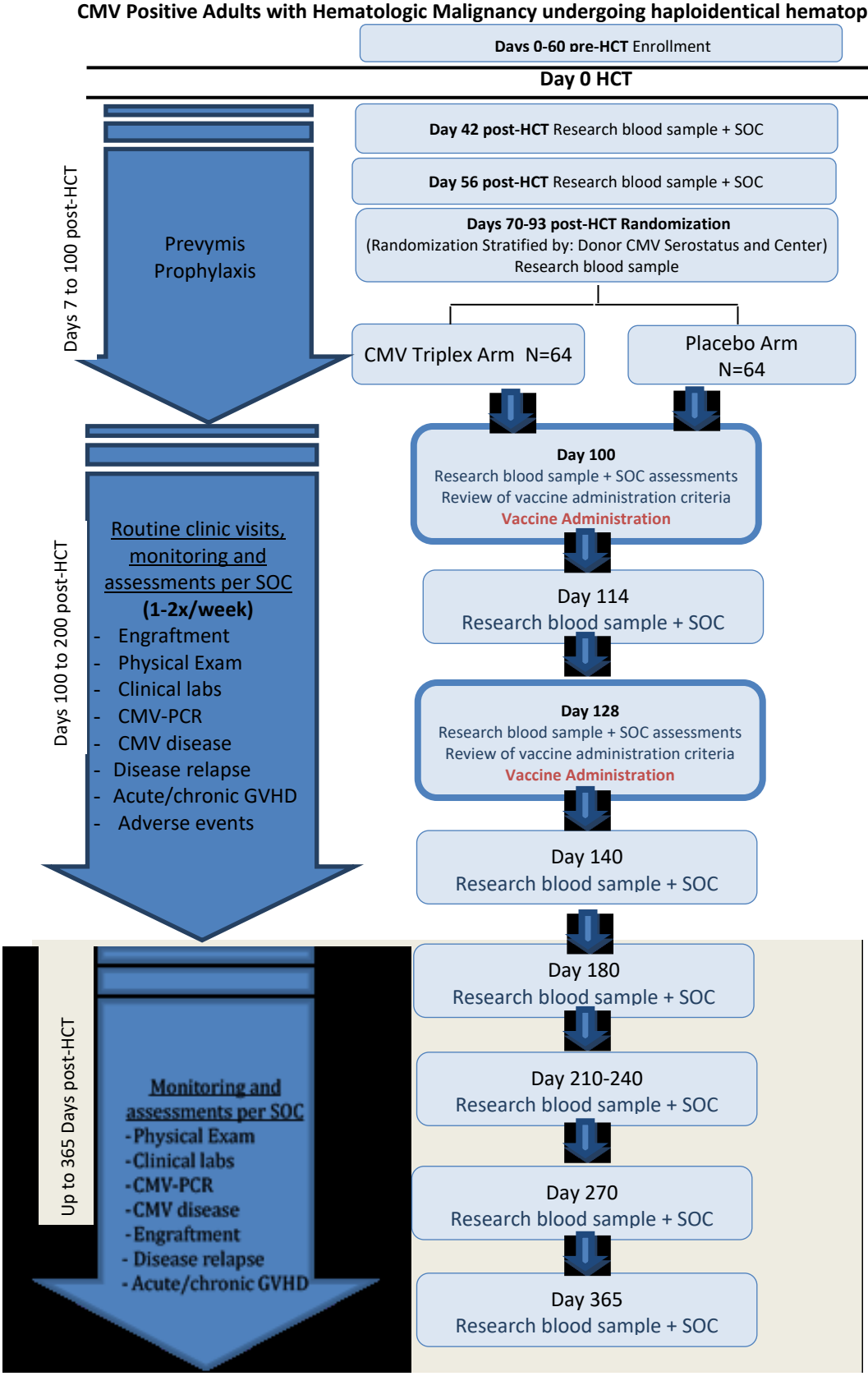
AGENT NSC# NSC# 745100

EXPERIMENTAL DESIGN SCHEMA

CMV Positive Adults with Hematologic Malignancy undergoing haploidentical hematopoietic stem cell transplant (HCT)



Enrollment:
- *d0-60 pre-HCT*

Randomization
- *Allocation communicated to pharmacy ~d70-93 post HCT.*

d7-d100
- *Prevymis administration per COH SOC*

d100 Evaluation for Vaccination
- *Participants who do not meet the d100 vaccine criteria are taken off study and replaced:*
- *Primary engraftment without secondary graft failure*
- *Absence of CMV disease and CMV viremia (qPCR ≤ 500 gc/ml)*
- *Disease has not relapsed*
- *No ongoing ≥ Grade 3 AEs*
- *Prednisone or equivalent must be <1 mg/kg/day for 7 days*
- *Only participants who receive a Day 100 vaccine are considered "randomized".*

Vaccine Administration
- *Vaccine must be administered within 4 hours of preparation start time.*
- *1 ml or 0.55 ml IM injection to upper arm.*

Disease Relapse
- *Participants who experience disease relapse are followed for survival only through d365.*

Clinical Care During Trial
- *Clinical care will be per SOC except:*
- *Vaccines are prohibited to d142 Post-HCT*
- *Prophylactic use of CMV IgG prohibited*
- *In vivo T-cell depleting agents are prohibited*
- *Prednisone or equivalent must be <1 or 1.5 mg/kg/day for 7 days prior to d100 and d128 to meet vaccine administration criteria*

PROTOCOL SYNOPSIS

Protocol Title:
A Phase II randomized, placebo-controlled, multicenter trial to evaluate the protective function of CMV-MVA Triplex vaccine in adult recipients of haploidentical hematopoietic stem cell transplant
Brief Protocol Title for the Lay Public (if applicable):
Triplex, a new vaccine designed to prevent cytomegalovirus infection after haploidentical stem cell transplantation
Sponsor, IND
COH under IND# 15792
Study Phase:
Phase II, Randomized, Blinded, Interventional
Participating Site:
City of Hope National Medical Center, Northside 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 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. Complementing antiviral prophylaxis 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 an immunogen 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]. 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 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, Triplex induced robust expansion of CMV-pp65, IE1 and IE2 CD8 and CD4 T cells in the healthy volunteers. There were no serious adverse events (SAE) or dose limiting toxicity (DLT). Following this,

a Phase II study to assess safety and efficacy of Triplex to protect against CMV reactivation and disease in allo-HCT patients was initiated. In the study vaccine arm also demonstrated significant increase in concentration of pp65-specific immune cells comparing to placebo, and maintained excellent tolerability with no significant difference in Grade >3 AEs between vaccine and placebo arms. The study also met the primary endpoint by showing clinically significant reduction in CMV events prior to d100 post-HCT.

The target of this new multi-center study is 128 evaluable patients (n=64 in each arm).

Anti-viral agent Prevymis was recently approved for CMV prophylaxis, however there is a significant risk of CMV reactivation that occurs after the completion of Prevymis administration [14]. This unmet need motivated the development of the protocol for the study to include a combination of Prevymis followed by Triplex vaccination. Complementing antiviral administration with a vaccine that harnesses the abundant native immune response to CMV may improve outcomes for HCT recipients [11]. In addition, this approach may protect against early reactivation in haplo HCT-R that was observed in the absence of Prevymis prophylaxis.

HaploHCT-donor (D) as a source of peripheral blood stem cells (PBSC) is the best option for finding a suitable donor when no conventional MR (matched related) or MU (matched unrelated) donor can be identified [15-18]. The incidence of CMV reactivation is far higher in haploHCT than in MRD or MUD HCT [19-23]. Consequently, these patients could benefit a from a vaccine preventing CMV infectious complications, especially after 18 weeks, when it has been shown that Prevymis prophylaxis declines in effectiveness [14].

Objectives:

The study will evaluate the Triplex vaccine in CMV+ adult patients undergoing haploHCT for hematological malignancy. This follows studies of the Triplex vaccine in lower risk MRD and MUD HCT where we have established tolerability (Trial NCT02506933).

Primary Objectives:

- To determine if CMV-MVA Triplex reduces the frequency of clinically significant CMV reactivation in CMV+ haploHCT adult recipients from when Prevymis prophylaxis is stopped at d100 until d180 post HCT. A clinically significant CMV reactivation encompasses use of antiviral treatment for CMV, or CMV disease by tissue histology (end-organ disease).

Secondary Objectives:

- To evaluate the safety and tolerability of Triplex up to d365 post-HCT in vaccinated HCT recipients by assessing the following: non-relapse mortality (NRM) at 180 days post-HCT, severe (grade 3-4) acute GVHD (aGVHD), and grade 3-4 AEs (CTCAE 5.0) probably or definitely related to the vaccination within 2 weeks from each vaccination at d180 post-HCT.
- To characterize CMV related events in recipients of Triplex compared to placebo up to d365 by assessing: time-to viremia (defined as number of days from d100 to the date of ≥625 IU/mL), duration of viremia, recurrence of viremia, incidence of late CMV viremia/disease (≥625 IU/mL >200 and ≤ 365 days post HCT), use of antiviral drugs other than Prevymis (triggered by clinically significant viremia), cumulative number of CMV specific antiviral treatment days.

- To evaluate the impact of Triplex on transplant related outcomes up to d365 post-HCT by assessing the incidence of acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, infections.
- To determine 1) if Triplex increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated, CMV seropositive HCT-recipients up to d365 post-HCT, 2) whether vaccination induces adaptive NK cell population changes, and increase in the highly cytotoxic memory $NKG2C^{+}$ NK cells up to d365 post-HCT, and 3) to compare GVHD biomarkers between the vaccine and placebo groups up to d365 post-HCT.
- To determine if immunity to 3 CMV antigens contained in the Triplex vaccine correlates with protection against CMV events, and if T-cell increases reflect vaccine response and exceed placebo immune response levels up to d365 post-HCT.

Study Design:

Overall design: This is a multisite (COH, DFCI, Northside), randomized, blinded, placebo-controlled, Phase II trial in CMV positive, adult haploHCT recipients. The study will evaluate: a) safety and reduction in the frequency of CMV reactivation after the end of a SOC treatment with Prevymis and b) increase in CMV cellular immunity in patients vaccinated with Triplex. This trial is designed to randomly allocate 151 (assuming 15% dropout rate) participants 1:1 to the Triplex or placebo arm. Both arms will be administered Prevymis from day ≤7 to day 100 post-HCT according to COH SOC. The accrual target is 64 evaluable patients for each arm.

Enrollment and randomization:

The trial is a double-blind randomized comparison of Triplex to placebo, powered to detect a clinically significant protective effect of vaccination on CMV reactivation, antiviral treatment and disease (CMV events) from d100 until d180 post-HCT. Patients will be enrolled d0-60 pre-HCT. Each adult haploHCT-R will be randomized to vaccine or placebo arm, stratified by donor CMV status and participating site. All eligible participants will receive vaccine dose or placebo on d100 post-HCT, with an identical second dose at d128 post-HCT. The design permits a direct comparison of placebo and vaccine with regard to the primary endpoint.

Administration of Triplex /placebo: Triplex vaccine or placebo is administered intramuscularly (IM) on d100 and d128 post-HCT.

Duration of participation: Study participation will be completed on d365 Post-HCT for all participants who receive a study-agent injection. Participants who do not receive an injection will discontinue any further follow-up once the determination is made that the vaccine will not be administered.

Stopping rules: One interim analysis with non-binding conclusion will be planned. Formal stopping rules for safety will be implemented, two major safety endpoints; non-relapse mortality (NRM) at d180 post-HCT, severe GVHD (grade 3-4 aGVHD, cGVHD requiring systemic therapy, secondary graft failure), 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:

The primary aims of this trial are to estimate the efficacy of Triplex in reducing the frequency of CMV reactivation after stopping Prevymis prophylaxis, and to further evaluate the safety and tolerability of Triplex vaccine in HCT recipients. Secondary endpoints will include additional safety, clinical and immunological parameters.

Primary endpoint:

1. Clinically significant CMV reactivation prompting antiviral therapy, or CMV disease (defined by histology) from d100 to d180 post-HCT.

Secondary endpoints:

1) Key safety endpoints: non-relapse mortality (NRM) at d365 post-HCT, severe (grade 3-4) acute GVHD, and grade 3-4 AEs probably/definitely related to the vaccination and MVA vector persistence.
2) CMV-related events: duration of viremia, duration of anti-CMV therapy, peak CMV PCR value, recurrence of CMV viremia, and incidence of late CMV reactivation or disease up to d365 post-HCT.
3) Transplant-related events: time to engraftment, incidence of aGVHD, chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, infections and overall survival up to d365 post-HCT.
4) Immunological function: levels and kinetics of CMV-specific T cell immunity, combined with immunophenotyping, and functional studies, NK phenotype and function (cytotoxicity and cytokine production) up to d365 post-HCT.

Sample Size:

The target is 128 randomized, evaluable subjects.

Projected enrollment: 151 enrolled patients should provide at least 64 per arm that are evaluable at d180. This is achievable in 10 years of accrual at the three sites.

Estimated Duration of the Study

10 years

Summary of Subject Eligibility Criteria:

Pre-HCT Inclusion Criteria

- Age 18 years and over
- Planned HCT for the treatment of hematologic malignancy (some exceptions including multiple myeloma)
- Planned related HCT with molecular 3/6 HLA donor allele matching (haploidentical)
- CMV seropositive (recipient)
- Seronegative for HIV, HCV and active HBV

Pre-HCT Exclusion Criteria

- Patients undergoing a second haploHCT 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
- 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
- Women who are pregnant or breast feeding

Investigational Product Dosage and Administration:

Participants will be randomized to receive either the 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 intramuscularly in the upper arm on d100 and d128 post-HCT. There are no dose modifications, although participants must meet vaccine administration criteria in order to receive the vaccine.

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 PBS containing 7.5% lactose.

Prevymis will be administered per COH SOC guidelines.

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. 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. The phenotypic ratios of CMV-specific T cells will be related to improvement in controlling CMV viremia. Characterization of highly cytotoxic memory NKG2C$^+$ NK cells, linked to CMV reactivation and critical for CMV adaptive immune response will be performed.

Statistical Considerations:

At COH, CMV reactivation is found in ~76% of CMV$^+$ haploHCT recipients by day 100 post-HCT (ASH abstract 2017 95% CI: 67-84%). At Northside hospital, of 127 consecutive CMV$^+$ haploHCT recipients who had not reactivated by day 28, 60% reactivated by d100 (unpublished). In Marty et al study among 102 high risk patients in Prevymis arm, 21 had clinically significant CMV infection at d100+, and 43 had clinically significant CMV infection through d168 post-HCT. CMV reactivation developed in 22 patients from d100+ to d168 among 81 patients who were event-free on d100+ (22/81=0.27). This trial will have 80% power at a one-sided 0.05 significance to detect reduction of CMV-reactivation from a conservatively assumed rate of 27% in Prevymis + placebo arm to 10% in Prevymis + vaccine arm at d180, attainable based on similar effect size in TransVax™ Phase 2 trial [24].

Analysis of CMV events: The primary statistical analyses will compare vaccine and placebo regarding subhazards of CMV events from d100 vaccine injection to d180. Events prior to injection will inform Kaplan-Meier estimates for both arms to obtain unbiased estimates of reactivation rates applicable to all transplanted subjects on each arm.

Non Relapse Mortality (NRM): NRM will be monitored as every 20th subject on the vaccine arm reaches the d100 evaluation point, i.e. d180 of follow-up or death. Stopping rules are specified in Table 4, which gives the maximum tolerated d180 NRM at each monitoring point. If NRM frequencies exceed these, then the trial will be suspended for safety review.

Severe GVHD/secondary graft failure: GVHD/secondary graft failure will be monitored as every 20th subject on the vaccine arm reaches the d100 evaluation point. The study will be interrupted for safety review by the external DMC if 6 or more of the first 15 recipients experience Grade 3-4 aGVHD. This would be a significant elevation from the expected maximum rate of 15% based on COH historical benchmark of 13.7% of haploHCT-R (M. Al-Malki et al, ASH 2017 Abstract).

Event-Free Survival (EFS) will be defined as the time from HCT to severe aGVHD, relapse, or death. EFS will be analyzed at the end of the study to produce a 90% 1-sided confidence limit on the hazard ratio. The EFS analysis thus provides due diligence in checking for a substantial adverse impact.

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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
COH	City of Hope
CRA	Clinical Research Associate
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
DMC	Data Monitoring Committee
ECG	Electrocardiogram
FDA	Food and Drug Administration
FC	Flow Cytometry
FOS	Foscarnet
GCP	Good Clinical Practice
GCV	Ganciclovir
GVHD	Graft versus host disease
LCL	Human B-lymphoblastoid cell lines
haploHCT	Haplo identical Hematopoietic Stem Cell Transplant
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
MRD	Matched related donor
MUD	Matched unrelated donor
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
Triplex	Cytomegalovirus-Modified Vaccinia Ankara (CMV-MVA) encoding 3 immunodominant CMV proteins
VAL	Valganciclovir

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

Primary Objectives:

- To determine if CMV-MVA Triplex reduces the frequency of clinically significant CMV reactivation in CMV+ haploHCT adult recipients from when Prevymis prophylaxis is stopped at d100 until d180 post HCT. A clinically significant CMV reactivation encompasses use of antiviral treatment for CMV, or CMV disease by tissue histology (end-organ disease).

Secondary Objectives:

- To evaluate the safety and tolerability of Triplex in vaccinated, haploHCT recipients by assessing the following: non-relapse mortality (NRM) at d180 post-HCT, severe (grade 3-4) acute GVHD, and grade 3-4 AEs (CTCAE 5.0) probably or definitely related to the vaccination within 2 weeks from each vaccination at d180 post-HCT.
- To characterize CMV related events in recipients of Triplex compared to placebo, by assessing time-to viremia (number of days from d100 to the date of ≥625 IU/mL), duration of viremia, recurrence of viremia, incidence of late CMV viremia/disease (≥625 IU/mL, >200 and ≤ 365 days post-HCT), use of antiviral drugs (triggered by clinically significant viremia), cumulative number of CMV specific antiviral treatment days.
- To evaluate the impact of Triplex on transplant related outcomes up to d365 post-HCT by assessing the incidence of acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, infections.
- To determine 1) if Triplex increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated, CMV seropositive HCT-recipients, 2) whether vaccination induces adaptive NK cell population changes, and increase in the highly cytotoxic memory $NKG2C^{+}$ NK cells, and 3) to compare GVHD biomarkers between the vaccine and placebo groups up to d365 post-HCT.
- To determine if immunity to 3 CMV antigens contained in the Triplex vaccine correlates with protection against CMV events, and if T-cell increases reflect vaccine response and exceed placebo immune response levels up to d365 post-HCT.

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 [25]. 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) [26-28]. 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 [29-31].

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, 32]. Despite this, CMV remains an important cause of mortality after HCT diminishing the full curative potential of this successful cancer therapy [33, 34]. 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, 35]. 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, 35]. 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 [36, 37]. When antivirals are stopped or when virus resistance occurs, the same disease symptoms appear; only frame shifted to ~d180 post-HCT [6, 38-40]. 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 [33]. Complementing prophylaxis using 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].

Cellular immune response to CMV in healthy adults: After primary infection, CMV persists under control of cell-mediated immune (CMI) surveillance [41-44]. Using whole genome overlapping peptide libraries, the targets of the healthy human T cell response to CMV were identified [25, 45]. These studies show that the main target for HLA Class 1-restricted T cells is the tegument protein UL83, referred to as pp65 [46-48]. However, immediate early proteins UL123 (IE1) and UL122 (IE2) also are prominent targets for CD8 T cell responses in healthy individuals and patients [25, 49-52]. These 3 targets have a profile that is consistent with protective immunity documented in HCT and SOT recipients [34, 53-55]. Phase 1 and 2 studies showed that pp65-ALVAC^{TM} stimulated primary immunity among CMV-negatives to levels approaching naturally CMV-positive individuals [56]. Similarly, AlphaVax^{TM} confirmed that property for pp65 and IE1 [57], and TransVax^{TM} DNA vaccine to a lesser extent [58]. These results provide a rationale for our choice of CMV pp65, IE1 and IE2 antigens to be incorporated into the vaccine.

CMV immune response in HCT Recipients: CMV interferes with proper immune function [58-65], 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 [66]. Several reports have associated protection from CMV disease in HCT recipients with CMV-specific $CD8^+$ T cell levels between 7-10/µL [59, 61, 67]. 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 [54, 55]. 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 [62]. Early clinical studies show that $CD8^+$ cytotoxic T lymphocyte (CTL) development is necessary to protect from CMV-IP in HCT recipients [3, 11, 68]. In patients who have measurable CMV viremia, expansion of $CD8^+$ T cells occurs, especially after viral reactivation [61, 63]. In addition, CMV disease occurred only in patients with low level CMV-tetramer$^+$ $CD8^+$ T cells [34, 60, 64]. Immunologic monitoring showed the benefit of CMV-positive donors even in the case of CMV-negative HCT recipients for long term CMV immune reconstitution [59, 65]. Cellular immunity in HCT recipients is augmented during CMV reactivation as shown by increased levels of functional CMV-specific $CD8^+$ T cells [34, 60, 61]. 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 [66, 69-72].

Vaccine Strategies against CMV for HCT recipients: The purpose of live viral vaccination is to induce both helper and cytotoxic immunity, leading to a durable memory response [73, 74]. 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 [68, 75]. 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 [76]. Alternative live viral approaches for CMV vaccines have focused on canarypox (ALVAC) expressing gB (UL55) which did not elicit significant antibody in CMV-negatives [77, 78], or ALVAC-UL83 (PP65) which stimulated robust cellular immunity in CMV-negatives equivalent to levels in natural CMV-positives [56]. Further studies with ALVAC-UL55 and purified soluble UL55 protein only revealed minimal efficacy, insufficient for licensure [79, 80]. Another approach using AlphaVax^{TM} expressing UL83, UL123 and UL55 [57] showed promise when used in healthy adults. However, it is unlikely that AlphaVax^{TM} can be accepted in HCT, since it is based on a recombinant VEE, a live virus that could propagate in humans [57]. TransVax^{TM} DNA vaccine vector expressing either UL55 or UL83 have been evaluated in animal models with good results [81-83]. When clinically evaluated, TransVax^{TM} vaccine, which requires multiple injections showed weak immune response in healthy adults, and failed to meet its primary endpoint of reduced GCV usage in HCT recipients [84, 85]. CMVPepVax, derived from the CMV-UL83 antigen showed remarkable safety and elicited vaccine driven immune responses when tested in healthy adults (IRB protocol #03121, NCT00712634) [86]. CMVPepVax was also being evaluated in HCT recipients (IRB protocol #12022, NCT01588015). In the HCT setting, the study indicated safety of injecting CMVPepVax in HCT recipients on d28 and d56 post-HCT, no increase in acute GVHD and reduced CMV reactivation associated with vaccine-stimulated immunity [87]. While CMVPepVax immunologic activity has shown promise, a limitation of HLA A*0201 restricted CMVPepVax is that it is solely active in the HLA A*0201 population, that only makes up ~30-40% of the at-risk HCT population. The Triplex vaccine is superior to DNA vaccines in stimulating potent immunogenicity. In addition, since it expresses whole CMV proteins it has broader recognition and greater applicability for HCT recipients than CMVPepVax.

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 [88, 89]. 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 [90-92]. 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 an immunogen 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 [93]. Multiple sites of foreign gene integration in MVA allow the virus to be modifed to express multiple full-length antigens. These facts motivated the development of vaccine candidates incorporating immunogenic targets of human CMV. Consistent with the ability to elicit responses against a recombinant MVA transgene product even in the presence 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 [94]. 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 [95], as well as in a Phase I HIV vaccine trial in which up to three recombinant MVA vaccinations were given eight weeks apart [96].

MVA for immunocompromised patients: Clinical trials were conducted in HIV infected and healthy adults [97] with a similar adverse event (AE) profiles in both groups. MVA was deemed safe to administer to HIV infected adults alone or alternating with a fowl pox vector [98]. In 2015 (NCT01571466), an MVA expressing multiple HIV antigens was given to patients who received disulfiram in a 3 injection regimen that was well tolerated without serious adverse event (SAE)[99]. Heavily immunocompromised patients who were both HIV and mycobacterium tuberculosis (MTB) positive were treated with an MVA expressing an MTB antigen (MVA85A) that was well tolerated with no vaccine-related SAE [100]. The same vaccine was also evaluated in adolescents (N=36) and 4-6-month infants (N=2797) in South Africa. There were no differences in AEs, and none of the SAEs were related to MVA85A [101, 102]. Critical to our program was an MVA vaccine trial in HCT-R [13]. There were 24 HCT patients who received MVA, 10 at low or high dose, and 4 received placebo. MVA was given twice separated by 28 days, the same time frame as in our completed trial in HCT-R (NCT02506933). There were 69 non-serious AEs, and all were unrelated to MVA. No subject had clinical symptoms, ECG findings, or troponin levels suggestive of vaccine-related myopericarditis. The patient cohort was comprised of equal numbers of allogeneic and autologous HCT-R, without significant differences in AEs and immunogenicity in both groups. Individuals who received prior smallpox vaccination were not at greater risk for AE. The conclusion is that HCT patients tolerate two doses of MVA with limited or no AEs. The Triplex approach using multiple CMV antigens should elicit broader immunity than previous single or dual antigen vaccines based on soluble protein, attenuated viruses, or DNA plasmids.

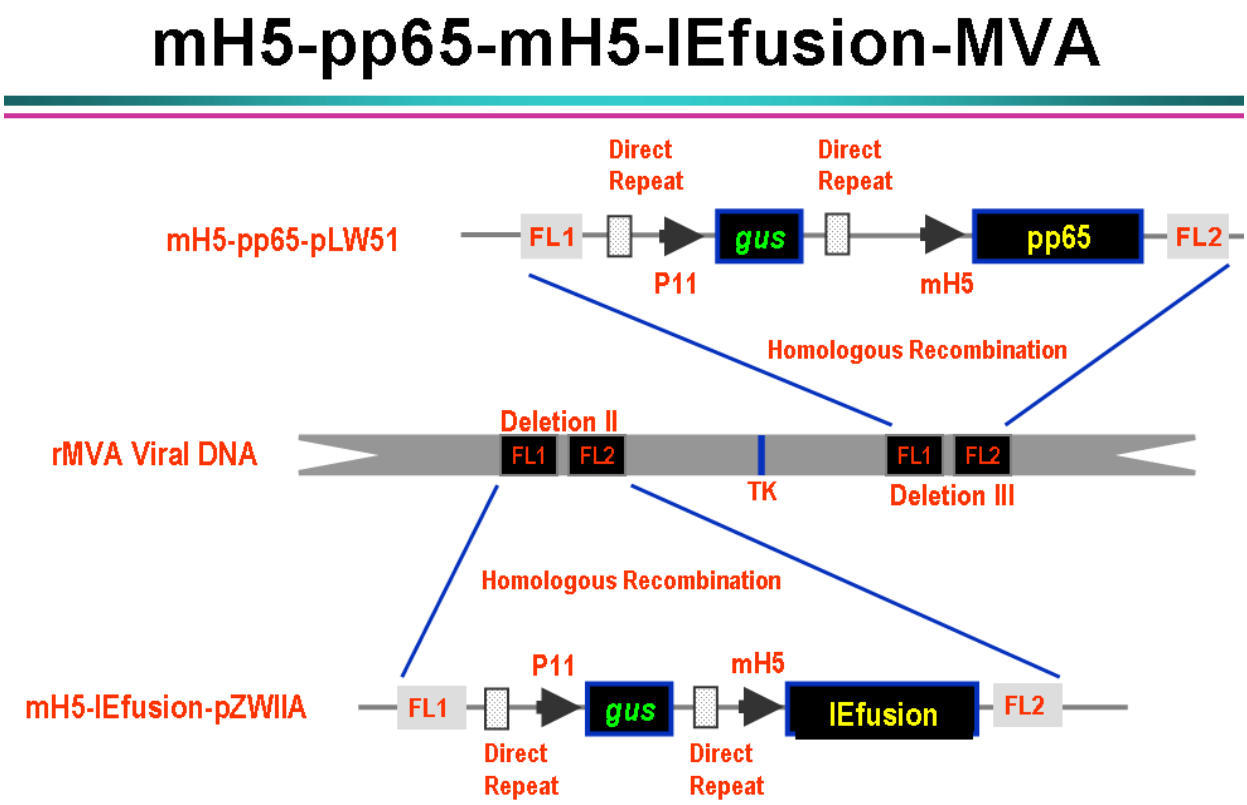
Construction, Expression and Function of 3-antigen MVA Vaccine against CMV:

3 CMV gene products, UL83, UL122, and UL123 have been selected as targets for cell mediated immune (CMI) responses [50, 103-105]. [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 [46-48, 106-109]. Recently, several reports have suggested that the CTL response to UL123 is as vigorous as the response to UL83 [25, 50-52, 110]. Each of the 3 antigens that are engineered into the multi-protein Triplex vaccine are immunodominant, and their aggregate CMI recognition should exceed 95% of most ethnic populations [103]. 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 [111]. 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 [112]. The strong cellular immune response induced by the Triplex vaccine does not neutralize CMV [113]. The majority of the CMV-neutralizing antibody response has been localized to the gB (UL55) and UL128 gene products [114-118]. As strong evidence that a humoral component impacts protection against CMV infection after HCT is lacking, gB has been omitted from this vaccine. Consequently, Triplex vaccine focuses on inducing a cell mediated response, which is 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 [56, 84, 85]. Investigators have defined regulatory activity of the UL123 protein, including trans-activating properties on various cellular promoters [119-121]. 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 [50]. The 406 aa protein has minimal transactivation activity [120, 121]. Most known CTL epitopes from UL123 are found in exon 4, including the identified HLA A*0201-restricted CTL epitopes [51, 52, 110, 122]. Exon5 of UL122 was fused in frame to exon4 of UL123 without modification [53].

Figure 1. Construction of Triplex vaccine



Insertion sites, promoter selection and permissive host cells for Triplex vaccine generation: MVA was derived by serial transfer 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 [53, 123, 124]. 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 GLP, pre-clinical studies, specific pathogen-free (SPF) CEF obtained from 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 [123]. 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 [125], and it provides a powerful boost to transgene expression without genomic instability [93, 126] (Figure 1).

2.2 Pre-Clinical and Clinical Evaluation of Triplex

2.2.1 Triplex vaccine

Evaluation of the immunogenicity of the Triplex in mouse models showed that the vaccine could stimulate primary immunity against all three CMV antigens (pp65, IE1 and IE2) in both the CD4 and CD8 T cell subsets [53, 127]. Furthermore, Triplex was evaluated *in vitro* for its ability to stimulate memory responses in PBMC from healthy adults and HCT recipients Interestingly, Triplex induced strong expansions of CMV-specific $CD4^+$ and $CD8^+$ T cell subsets in both healthy adults and in patients within 6 months of receiving HCT [53, 127].

Based on the body of pre-clinical and *in vitro* results the FDA (BB-IND #15792) granted allowance to proceed with human studies. A Phase Ib clinical trial to evaluate the safety and biological efficacy of Triplex vaccine in healthy volunteers, with or without prior immunity to CMV and vaccinia was initiated.

2.2.2 Phase Ib clinical trial in healthy adults (IRB 08173, NCT01941056)

Triplex vaccine was evaluated in a Phase Ib clinical trial, conducted in healthy adults to assess safety and immune response. To establish tolerability of 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 1.0mL volume in the upper non-dominant arm, each at the same dose over a 4-week period.

2.2.3 Safety of Triplex vaccine

Twenty-four healthy volunteers (HV) were vaccinated with Triplex Vaccine. Three doses of vaccine were evaluated:

DL1: 1×10^7 pfu

DL2: 5×10^7 pfu

DL3: 5×10^8 pfu

Some AEs were reported at each dose level, but the majority were grade 1-2 (see Table 1). The trial has been completed, with none of the patients experiencing SAEs or DLT, or withdrawing from the study [128]. Thus, the first in human study of Triplex vaccine has met the primary objective, showing excellent tolerability (Table 1).

Grade 1-2

Attribution	Dose Level	Category	Adverse Event	Grade 1~2
Unlikely, Unrelated	Arm 1 - Dose Level 1		AST (SGOT) (CHEMISTRIES)	1
			Hypertension (CARDIOVASCULAR)	1
	Arm 2 - Dose Level 2		Cough (RESPIRATORY)	1
			Hemoglobin (HEMATOLOGY)	1
	Arm 3 - Dose Level 3		Fever, Oral (SYSTEMIC)	1
			Hypercalcemia (corrected for albumin) (C	1

Attribution	Dose Level	Category	Adverse Event	Grade 1~2
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)	
			Fatigue (SYSTEMIC)	8
			Headache (SYSTEMIC)	5
			Hypertension (CARDIOVASCULAR)	1
			Induration (SKIN)	1
			Myalgia (MUSCULOSKELETAL)	7
			Nausea (GASTROINTESTINAL)	2
			Paresthesia (burning, tingling, etc.) (N	1

Grade 3-4

Dose Level	Category	Adverse Event	Grade 3
Arm 3 - Dose Level 3		Erythema (SKIN)	1

Table 1. AEs in healthy adults trial (Phase Ib trial, Protocol 08173)
*There were 8 patients dosed at each dose level (DL1, DL2, and DL3).

Cardiac 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 [129]. No change in ECG and troponin levels was reported for all Triplex vaccinated volunteers and no other cardiac AE were reported (data from Phase 1b and Phase 2 studies).

Persistence of Viral DNA: Persistence of the Triplex vaccine in healthy volunteers was monitored in accordance with FDA recommendations. A real-time PCR approach employing separate sets of primers for

the MVA backbone and the CMV insert genes utilizing TaqMan™ reagents was used. 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 detectable in blood specimens. Persistence of the Triplex vaccine was monitored in healthy volunteers after administration of both doses of vaccine. DNA from the dose level 1 and 2 cohorts showed undetectable levels of MVA DNA. In the Dose Level 3 cohort, low levels of MVA DNA, above the assay detection limit, were detected in 2/8 of the vaccinated subjects at d90 post vaccination. Considering the high doses of Triplex vaccine administered to this cohort, the low level of MVA DNA detected, at a single time point, is not a cause for concern.

Triplex Induced Immune Responses – secondary endpoint

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 [130]. Triplex vaccine driven immune responses were robust in DL1 (N=8) and DL2 (N=8) healthy volunteers. 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. 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 ($p = 0.003$) [128].

2.3 Triplex Vaccination in Allo-HCT

We obtained FDA approval for a trial entitled "A Phase 2 randomized placebo-controlled trial to evaluate the protective function of a Triplex vaccine in recipients of an allogeneic HCT (NCT 02506933)." Target accrual of the multi-center study (COH, DFCI, and MD Anderson) was 102 evaluable patients (n=51 each arm). Primary objectives were assessing safety and tolerability of Triplex (NRM at d100 post-HCT, severe aGVHD, Grade 3-4 AEs related vaccination). In addition, we assessed whether Triplex reduces the frequency of CMV events, defined as CMV reactivation (DNAemia >625 IU/ml by qPCR), viremia treated by antivirals, or detection of CMV by tissue histology (end-organ disease). Secondary objectives included measurement of viremia and treatment duration, HCT-related outcomes (aGVHD, chronic GVHD, relapse, NRM, and infections). Figure 2 shows the Clinical Trial schema for this study.

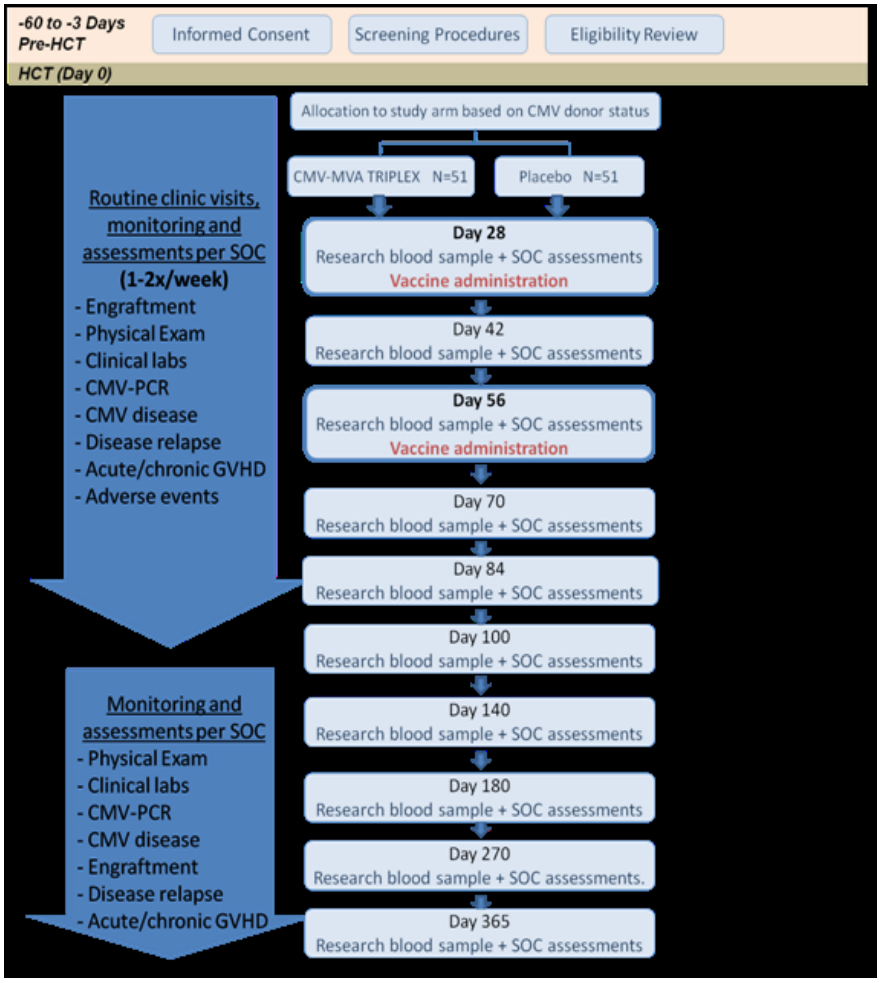


Figure 2. Schema for Phase II Triplex study IRB 14295 (NCT02506933)

Schema for the Phase 2 randomized, placebo-controlled multicenter trial to evaluate the protective function of Triplex vaccine in recipients of an allogeneic hematopoietic stem cell transplant.

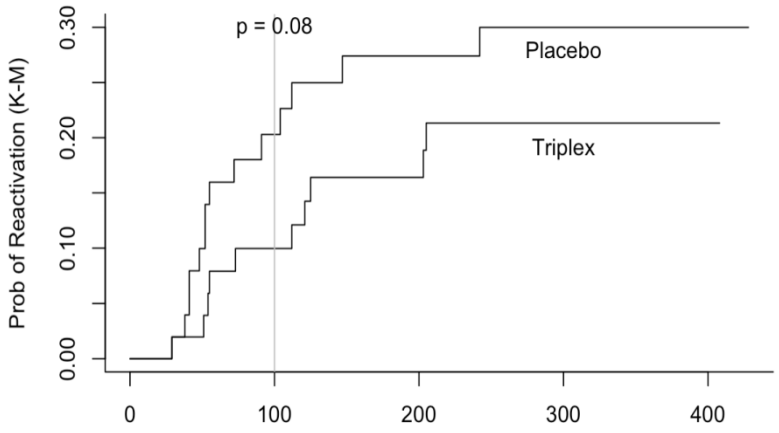


Figure 3. Reactivation data from Phase II Triplex trial IRB 14295 (NCT02506933)

Kaplan-Meier estimates of cumulative incidence, as estimated probability of reactivation as a function of day post-HCT. The p-value shown is for the protocol-specified test comparing CMV events in Triplex and placebo arms at d100.

Figure 3 shows Kaplan-Meier estimates of compiled data from 102 patients (24 CMV events, including 14 in placebo and 10 in vaccine arms). In this study Triplex met the protocol-specified primary endpoint – clinically significant reduction in CMV events prior to d100 post-HCT, even though fewer CMV events occurred in this trial than were anticipated in trial design. There was no statistical difference in NRM, severe aGVHD or Grade >3 AE between placebo and vaccine arms.

We have also conducted immunologic assessments of patients on this double blinded trial. Strong immunity to CMV was apparent shortly after the first injection. There was a substantial increase in pp65 $CD8^+$ T-cells in the vaccine arm compared to placebo by d42, and d56 for IE1 and IE2 which continued to rise through d100. CD4 and CD8 pp65-specific T cell concentration was higher in vaccine arm across all vaccination times (data available on request). This is associated with protective immunity in published natural history studies of immune reconstitution [61, 67].

2.4 Triplex in HaploHCT

HaploHCT was designed to overcome major HLA barriers in HCT by using alternative donors when fully HLA-matched donors were unavailable, particularly for those who are racial/ethnic minorities [131]. Major HLA mismatches can lead to severe GVHD or graft rejection. T cell depletion (TCD) can minimize GVHD, but can lead to unacceptable relapse, infection, graft failure, and non-relapse mortality (NRM) [132]. Current regimens which include post-transplant cyclophosphamide (PTCY) are successful in reducing morbidity and mortality on par with MRD and MUD HCT [133, 134]. PTCY was evaluated clinically as a two-step approach in which it was administered in both the conditioning regimen and post-HCT on d3 and d4 (50 mg/kg) with improved clinical outcomes [135]. Utilizing more intensive myeloablative conditioning (MA) and separating mature T cells from $CD34^+$ stem cells (to avoid exposure of $CD34^+$ cells to PTCY), can increase 2 year survival [136, 137]. In older patients intolerant of MA, reduced intensity conditioning (RIC) was employed, followed by PTCY. This results in outcomes similar to MA, and has generalized the use of PTCY to most leukemia patients [138, 139]. The Hopkins approach has now been expanded to include greater MA conditioning regimens including total body irradiation (TBI) or chemotherapy-based and use of GCSF-mobilized PBSC. In summary, haploHCT has outcomes similar to traditional HCT, with the added benefit of expanding the donor pool.

CMV infection in haploHCT-R. A serious complication of haploHCT is slow immune reconstitution leading to multiple viral infections. While PTCY lowers GVHD rates, it contributes to delayed immune reconstitution and infectious complications which are important causes of morbidity and mortality. The immunosuppressive therapy for GVHD prophylaxis antagonizes the immune reconstitution that protects against infections. GVHD treatment, or GVHD itself, slows immune recovery and makes patients susceptible to opportunistic infections for several months after haploHCT. Reactivation of viruses such as CMV, EBV, HSV or VZV can lead to symptomatic disease [22].

Antiviral agent Prevymis has been approved by the FDA for all CMV seropositive HCT recipients to minimize the chance of CMV reactivation and disease development and the use of Prevymis for this purpose is now adopted into the HCT SOP. Unfortunately, CMV-specific T cell immune reconstitution has not been described in patients receiving Prevymis, and late CMV reactivation after stopping Prevymis on d100 remains a major clinical problem [14]. This problem can be potentially overcome by the sequential use of Prevymis and Triplex. We are intending to study this highly novel treatment strategy combining the benefits of Prevymis and Triplex vaccine developed by COH. This combination has no overlapping toxicities, and is potentially synergistic, or at least should possess additive protective effect for CMV infection.

The importance of CMV-specific memory T cells is illustrated by the fact that HCT recipients with CMV-positive donors have better outcomes (reduced NRM, improved survival) than those with CMV- donors. This is attributed to donor-derived CMV-specific T cells allowing rapid CMV-specific immune reconstitution [140].

One attribute of the Triplex vaccine is its ability to enhance CMV-specific T cells, particularly those from the TEM (Terminal-Effector Memory) and TEMRA (TEM-CD45RA) subsets that are associated with protection against sequelae of CMV infection. It was understood that TCD haploHCT made the patient vulnerable to multiple infections, including CMV [132]. Earlier attempts to overcome deficits in T cell reconstitution for CMV utilized adoptive transfer of CMV-specific $CD4^+$ T cell clones which was an effective strategy to limit mortality associated with CMV infection [141]. Nonetheless, obstacles such as requirements for sophisticated devices to isolate and separate CMV-specific T cells have limited the broad applicability of this approach. An alternative GVHD prophylaxis using PTCy for haploHCT has been advocated by the Johns Hopkins group [16, 142]. The average time of CMV reactivation is d39 in a major Italian study that mirrors other haploHCT studies, which is consistent with the timing in conventional HCT (d42-45) [143]. There was a 54% CMV reactivation rate in 70 CMV+ or CMV- lymphoma patients after unmanipulated haploHCT using PTCy. Most CMV reactivations occurred post-d31 and up to d100 in line with other haploHCT series [19]. Despite the premise that PTCy retains memory T cells not reacting against host allo-antigens, an average of ~60-80% of CMV-P HCT-R still have CMV reactivation. This level of risk is an ideal scenario for evaluation of the Triplex vaccine which could substantially reduce the rate of reactivation and lessen the probability of uncontrolled viremia or other symptomatic CMV infections such as end organ disease.

2.5 HaploHCT Study Overview

This randomized, blinded, placebo controlled, multi-site Phase II safety and efficacy trial will be conducted at COH, Northside hospital and DFCI. This study has been designed to have sufficient statistical power for testing clinically significant endpoints. In particular, the efficacy of Triplex in protecting against CMV reactivation and disease in haploHCT recipients (CMV positive) after d100 post-HCT (d100-d180) who are at risk for CMV complications after the completion of Prevymis prophylaxis [59].

Primary hypothesis:

- The CMV reactivation that leads to antiviral treatment or CMV disease by histology (end organ disease) for haploHCT-R will occur with less frequency on the Triplex vaccine arm than on the placebo

arm.

S*econdary hypothesis*:

- CMV protective immunity after d100 post-HCT and completion of Prevymis prophylaxis will be safely enhanced in haploHCT recipients receiving two Triplex vaccine injections leading to reduced frequency of CMV reactivation and its recurrence, antiviral use and its duration, and incidence of CMV disease (CMV events).
- Immunity to 3 CMV antigens contained in the Triplex vaccine will correlate with protection against CMV events, and T-cell increases will reflect vaccine responses and exceed placebo immune response levels.

This trial aims to treat 128 haploHCT-R^+, randomized to the vaccine (N=64) or placebo arm (N=64). Because approximately 15% of enrolled participants may fail to meet post-HCT vaccine administration criteria, the total study accrual is expected to be ~151 participants. Participants meeting vaccine administration criteria will receive injections of either Triplex or placebo on d100 and d128 post-HCT.

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 d100 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 time points per SOC. Rigorous stopping rules will be implemented, and will include three major safety endpoints:

- non-relapse mortality (NRM) at d180 post HCT
- severe (grade 3-4) acute GVHD between d100 and d180
- grade 3-4 AEs related to the vaccination within 2 weeks from each vaccination

CMV-specific immunogenicity (included in the secondary endpoints) will be evaluated in all participants from d100 until d365 post-HCT according to the study calendar. Immunologic studies will include monitoring the levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated haploHCT-R, combined with immunophenotyping studies [144, 145]. 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 published Phase Ib study (COH IRB 08173, NCT01941056), 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 [34].

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:

<u>Informed Consent and Willingness to Participate</u>

___ 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+ years.

Nature of Illness and Transplant Related Criteria

___ 4. Planned PBSC or BM 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 judged appropriate by the clinical PIs, 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 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 haploHCT are not eligible (patients who have undergone a previous autologous HCT are eligible).

___ 5. Patients receiving myeloablative (MA) or reduced intensity conditioning (RIC) are allowed

___ 6. CMV seropositive (recipient)

___ 7. Planned related HCT with molecular 3/6 (haploidentical) intermediate/high resolution HLA donor allele matching

___ 8. Planned HCT with minimal to no-T cell depletion of graft

___ 9. Conditioning and immunosuppressive regimens according to institutional guidelines are permitted

Clinical laboratory parameters

___ 10. Negative serum or urine β-HCG test (female patient of childbearing potential only) within two weeks of registration.

___ 11. Seronegative for HIV, HCV and active HBV (Surface Antigen Negative) within 2 months of registration and no history of disseminated cutaneous HPV related disease.

Child Bearing Potential

___ 12. 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 d90 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 d70 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 antigen injections

___ 6. Alemtuzumab or any equivalent in vivo T-cell depleting agent (or $CD34^+$ selection)

___ 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 EXCEPT Prevymis prophylaxis (prior to d100)

___ 9. Conditioning regimens d30 prior to trial participation and up to d180 post-HCT

___ 10. Disease-based radiation therapy (not total body irradiation)

___ 11. 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.

___ 12. Other medications that might interfere with the evaluation of the investigational product (see Prohibited Medications, Section 5.6)

Other illnesses or conditions

___ 13. Patients with active autoimmune conditions requiring systemic immunosuppressive therapy within the previous 5 years are not eligible

___ 14. Patients considered by PIs/Protocol team to have a complicated prior therapy or HCT regimen, or who have a low survival probability (e.g., refractory leukemia and/or undergoing 2^{nd} HCT)

___ 15. Poor risk disease/disease status including: CML in blast crisis, AML/ALL beyond 2^{nd} remission, multiple myeloma, and aplastic anemia

___ 16. Pregnant women and women who are lactating. 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.

___ 17. 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

___ 18. 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 (d60 to d0 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)
   - 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 OnCore for MD Anderson and DFCI participants (the COH CRC will directly accession into OnCore), 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

Patients will be randomized from d70-93 post-HCT with the goal of evenly distributing participants into 2 arms (64 patients to the vaccine arm and 64 the placebo arm). 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 table, stratified by donor CMV serostatus and center (generated by the trial Biostatistician), to assign registered participants to the Triplex or placebo arm. The treatment assignment will be generated and provided to pharmacists in advance (usually ~d70-93 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 (Triplex or placebo) on d100 will be considered “randomized”.

4.4 Emergency Unblinding Procedures

The COH IDS which will be authorized in an emergency to break the code and inform the responsible party at the DFCI Investigational Pharmacy and similarly at the Northside Investigational Pharmacy. Consequently, the HCT-R’s physician will not have access to the randomization assignment on this trial.

Emergency un-blinding will occur if a patient on this study develops a life-threatening toxicity or SAE and the participant’s physician 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 unblind patient’s treatment.

In this very unlikely event, the PMT will determine if and how the unblinding 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 external DMC as per COH institutional requirements. The date and reason for unblinding 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 Prevymis administration from d7 until d100 post HCT (per COH SOC guidelines) and vaccine administration on d100 and d128 post HCT of either Triplex (N=64) or placebo (N=64), depending on participant randomized treatment assignment.

Participants will receive vaccine administration only 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 d100 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 d365 post-HCT.

Time intervals 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 via syringe covered for blinding purposes, but known to the DCC coordinators, study monitors, auditors, pharmacy personnel, and the statistician. The vaccine will be administered by a CRU nurse who is not involved in other protocol-required procedures, does not provide day-to-day medical care to the patients in the trial, and is independent of investigators conducting the trial. 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 and 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. An un-blinded PMT contact for Pharmacy will be designated. 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. These procedures of maintaining blinded randomization are based on successful implementation of identical procedures in completed clinical trial IRB#14295.

Unblinding to know the randomization status will be permissible when all enrolled patients have finished the planned follow up and the study is closed to accrual.

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

Trial participants will be quantitatively monitored for viremia, by plasma qPCR test once every two weeks (+/- 5d) from d100 to d180. CMV monitoring will use standard qPCR clinical laboratory methods to evaluate CMV viral load and possible vaccine failure. The methods used at the 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; 250 gc/ml = 625 IU/mL).

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

Northside: Qiagen qCMV PCR, detection limit 100 CMV copies mL (conversion factor: LOG IU = [1.0264*(LOG copies/ml)] – 0.0156, anti-LOG equals IU/mL).

Based on available information these methods are considered sufficiently similar. However, selected samples with measured viral load ≥625 IU/ml from DFCI and Northside study subjects will be stored for batch testing at COH to confirm equivalence of methods at external sites with COH method (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. Late CMV disease typically occurs around 6 months post-HCT, and will be monitored. When clinically indicated and per SOC, CMV disease will be assessed. 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 ≥625 IU/mL will be evaluated by the treating investigator in conjunction with the blinded PMT before a determination is made. Viremia diagnosed using qPCR [146] of ≥625 IU/mL will be treated with antivirals according to institutional guidelines.

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 d365.

5.3.6 Clinical laboratory chemistry, hematology 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.

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 v.5.0)

All adverse events will be assessed using NCI CTCAE v. 5.0, which can be found at the following link: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf. AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study injections, described as "expected" or "unexpected problem", and analyzed from d100 injection through d365 observation period and accompanied by a written description to meet IRB requirements. The CTCAE v5.0 toxicity scale will be used to characterize expected AEs. 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 d180
- Highest grade events that occur within 2 weeks from each vaccination
- All grade 3/4/5 and highest grade events will be reported from d100 through d180
- All serious adverse events will be reported to the sponsor from d100 through d180
- After d100, 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 condition up to d0, but prior to stem cell infusion. Baseline symptoms are defined as any symptoms on d100 prior to vaccination.

5.3.9 Concomitant medications

All antiviral medications, immunosuppressive agents, prednisone, and other anti-cancer therapy or maintenance therapy taken or administered during the trial will be documented in the subject's clinical/hospital record, using COH, Northside and DFCI 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 becomes ineligible prior to the d100 vaccine (such participants are not randomized and are replaced, with ineligibility data recorded)
- 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 d100 vaccine administration

Participants who do not meet criteria for d100 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 d365 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 d128 vaccine (if not already administered),
- may elect to continue to be monitored for survival (including reason for mortality) until d365 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 d365 post-HCT. Study participation will be completed on d365 post-HCT.

5.5.5 Long-term Follow Up

Using a specific long-term follow-up protocol at COH (IRB05116, Dr. Armenian, PI) and similar schemes at DFCI and Northside that will be described in their clinical protocol, enrolled patients will be consented to participate in an extended follow-up of 3 years post-HCT. This extended period of observation will help understand differences in outcome in vaccine and placebo cohorts. Long-term immune response to CMV and survival will be compared in these two subgroups.

5.6 Supportive Care, Other Concomitant Therapy, Prohibited Medications

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

1. No other investigational agents may be given to patients
2. 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 Triplex.
3. Preemptive therapy with CMV immunoglobulin or antivirals (GCV/VAL, FOS, Cidofovir, CMX-001) is not allowed following HCT, EXCEPT:

   1) Prevymis administration between d7 and d100 post-HCT according to COH SOC guidelines.

   2) GCV/VAL, FOS, Cidofovir, CMX-001 may be used according to institutional SOC for preemptive management of CMV viremia, but vaccination is only allowed no less than a month after administration of antivirals is completed. In general, therapy should not commence until after CMV ≥625 IU/mL. For preemptive therapy when qPCR<500 IU, the study PIs are to be consulted.
4. 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.
5. Medications that might interfere with the evaluation of the investigational product are prohibited up to 14 days after the second vaccination (d142 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 prior to vaccinations).

6.0 VACCINE ADMINISTRATION CRITERIA

6.1 D100 Post-HCT Vaccine Administration Criteria

If the criteria are not clear cut, the treating clinician/Site PIs will make a determination. This consultation should be documented. Eligibility for d100 study-agent injection will be assessed at approximately d 70 post-haploHCT. Randomization will occur coincident with assessment of eligibility for d100 injection. At d100, a first injection shall be administered if the following criteria are met:

1. NOT experienced ≥ Grade 3+ GVHD within past 7 days
2. Disease has NOT relapsed since HCT
3. Successful primary engraftment WITHOUT secondary graft failure
4. NO ongoing post-HCT ≥Grade 3 non-hem AE's (exceptions: glucose intolerance, cholesterol, triglyceride, and hyperglycemia)
5. Negative for CMV viremia: CMV qPCR ≤625 IU/mL from samples collected within the past 7 days
6. Negative for CMV end organ disease (biopsy proven) post-HCT
7. All prednisone (or equivalent) doses within the past 7 days were ≤1 mg/kg/day
8. NOT received any prohibited medications (Section 5.6), EXCEPT antiviral use if the administration was completed at least 1 month before vaccination
9. Negative pregnancy test result for females of child bearing potential
10. No active pathogen infection defined as positive blood cultures within 72 hours of injection or radiographic changes consistent with infection
11. Platelet count of $\geq$50,000/ml (transfusions to achieve this level are acceptable)

The DCC should be promptly notified of registered participants who fail d100 vaccine eligibility criteria.

6.2 D128 post-HCT Vaccine Administration Criteria

Prior to d128, patients will be reviewed for eligibility. D128 vaccine shall be administered if all the following are met:

- NOT experienced >Grade 2 GVHD since d100 vaccination
- NO Grade 3 GVHD within the past 7 days
- Disease has NOT relapsed since HCT
- NO secondary graft failure (ANC<500/m^3)
- NO ongoing post-HCT ≥ Grade 2 non-hem AE's (exceptions: grade 3 glucose intolerance, cholesterol, triglyceride and hyperglycemia)
- All prednisone doses (or equivalent) within the past 7 days were ≤ 1 mg/kg/day
- NOT received any prohibited medications (Section 5.6)
- Negative pregnancy test result for females of child bearing potential

CMV reactivations/anti-CMV treatment between d100 and d128 do not disqualify patients from d128 injection.

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. Data and safety monitoring for the protocol will be performed in accordance with the NCI-approved City of Hope Data and Safety Monitoring Plan (DSMP) for Cancer Center Trials. In accordance with the NCI Comprehensive Cancer Center rules, safety monitoring on subsite patients will be performed locally. Data and safety monitoring will be performed in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP) for Cancer Center Trials. Similarly for DFCI and Northside Hospital, the COH DSMP will be followed. These comprehensive plans describe risk assignment, AE reporting procedures and the data and safety monitoring procedures overseen by the respective IRBs and by the COH and/or external DMC.

7.1 Monitoring and Personnel Responsible (PMT)

The PMT will consist of the Study PI, Site PIs, collaborating investigators, Biostatistician and CRC/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 evaluation by the Independent DMC prior to DMC meetings.

7.2 AE and UP Definitions

The research team is responsible for classifying AEs and UPs as defined in the relevant regulations and reporting to all applicable parties, including but not limited to the COH IRB, DSMC, Food and Drug Administration (FDA), National Institutes of Health (NIH) and other collaborators, e.g., pharmaceutical companies. The research team is responsible for the continued monitoring and tracking of all AEs in order to ensure non-reportable events are reviewed and monitored and do not rise to a reporting level.

7.2.1 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.

7.2.2 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.

7.2.3 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.

7.2.4 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 d180 post-HCT 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).

7.2.5 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.

7.2.6 Assessment of Adverse Events

The site Investigator will be responsible for determining the event name, and assessing the severity (i.e. grade), expectedness, and attribution of all adverse events as applicable per the City of Hope Clinical Research Adverse Event and Unanticipated Problem policy. Adverse events will be characterized using the descriptions and grading scales found NCI CTCAE v5.0. A copy of the scale can be found at:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm.

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

- **Unrelated –** The event is clearly NOT related to study treatment, and is clearly related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant medications administered to the participant.
- **Unlikely –** The event is unlikely related to the study treatment, and is most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible –** The event may be related to study treatment, as it 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 is most likely related to the study treatment, as it follows a reasonable temporal sequence from the time of drug administration and a known response pattern to the study drug, and is unlikely related to the participant's clinical state, other therapeutic interventions, or concomitant drugs.

- **Definite –** The event is clearly related to the study treatment, as it follows a reasonable temporal sequence from the time of drug administration and a known response pattern to the study drug, and is not reasonably explained by other factors such as the participant's condition, therapeutic interventions, or concomitant drugs.

7.3 Routine Reporting of Non-Serious 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:

- Highest grade events in the reporting period considered possibly, probably or definitely related to study agent
- Highest grade events regardless of attribution
- All serious adverse events from d100 to d180

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, comorbidities)

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 d30 post-study drug assessment. The Coordinating Center should be consulted prior to ending the follow-up of events that have stabilized.

7.4 Expedited Reporting of SAEs and Unanticipated Problems (UPs) by Site Investigators to the COH Regulatory Committees

Serious Adverse Events that require expedited reporting and unanticipated problems will be reported according to the approved City of Hope Clinical Research Adverse Event and Unanticipated Problem policy. Reporting of all UPs and SAEs will begin from the first vaccine administration till d30, and must be followed until the 30 days after the last dose of vaccine. Follow-up SAE 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.

Non-COH Sites:

Serious Adverse Events meeting the criteria specified in the City of Hope Clinical Research Adverse Event and Unanticipated Problem policy will be reported to the Data Coordinating Center (DCC) and PI within **24 hours** of notification that the event occurred.

Procedure for reporting SAEs/UPs to the COH DCC:

1. Sites are to report to their local IRB per their site's specific institutional and IRB guidelines. As soon as possible, non-COH sites will provide to the COH DCC copies of the IRB submission and corresponding IRB response.
2. Document/describe the SAE/UP on each of the following:
   a. MedWatch 3500A: Downloadable form at http://www.fda.gov/medwatch/getforms.htm
   b. UP/SAE Coversheet: A modifiable Microsoft Word document is also available from the DCC. An electronic signature on the document will be accepted.

3. Scan and email above documents to study Principal Investigator (rnakamura@coh.org) and DCC@coh.org with the subject title as "COH IRB 19065 SAE". If an email receipt from DCC personnel is not received within one working day, please email DCC@COH.org.

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

7.4.1 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 d30 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.

7.4.2 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)].

7.5 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 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 one month after the following occurs:

- approx 32 patients reach d180;

- approx 64 patients reach d180;
- approx 96 patients reach d180;
- At study conclusion (optional final DMC meeting).

After any NRM within 180 days post-HCT, 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.

7.6 Toxicities of Triplex

7.6.1 Expected (known) toxicities of Triplex

The potential risks associated with Triplex injection includes pain, swelling, and erythema at the injection site, and the potential for bleeding into the site, with possible later abscess, though these risks appear to be minimal based on our prior experience. In the completed Phase 2 clinical trial (NCT02506933), in alloHCT, the following AEs were attributable to the injection beyond Grade 2 in 11 patients immunized twice (Table 2).

Table 2. Grade 3-4 attributable adverse events observed in IRB 14295 (NCT02506933)

Within 2 weeks after d28 injection

MEDDRA term	Max.Grade	Attribution	Placebo	Vaccine
Alanine aminotransferase increased	3	Probable	1	0
Anemia	3	Possible	1	0
Lymphocyte count decreased	4	Possible	0	1
Neutrophil count decreased	3	Possible	0	1
Platelet count decreased	3	Possible	1	0
White blood cell decreased	3	Possible	0	1

Within 2 weeks after d56 injection

MEDDRA term	Max.Grade	Attribution	Placebo	Vaccine
Alanine aminotransferase increased	3	Possible	2	0

7.7 Toxicities to the placebo

7.7.1 Expected (known) toxicities to placebo

There are no expected (known) toxicities associated with administering the placebo.

7.8 Risks from Vaccinations and Blood Draws

In the event of an AE related to vaccination or blood draws, emergency medical care to treat the injury will be provided. Patients will be treated at the HCT unit by highly skilled medical staff. Treatment of such patients is the responsibility of the HCT physician who will consult with either Dr. Nakamura or the local PI in regard to appropriate medication and intervention.

7.8.1 Vaccinations

Vaccinations will be performed in the appropriate CRU at COH, DFCI or Northside. Injection procedure will conform to guidance on vaccination of persons with bleeding problems of the Committee on Infectious Diseases. Patients will be observed in the clinic after vaccination. There will be telephone contact with the

CRA and the HCT nurse coordinator assigned to this study.

7.8.2 Blood draws

HCT-R blood draws will take place at either inpatient or outpatient facilities. To minimize risks, draws are performed through a central catheter at times when routine blood tests are done and by nurses highly skilled in this procedure (>1500 HCT yearly among all three sites). Peripheral blood collections later in the study, when the central line is removed, will be limited to monthly or bimonthly.

To avoid lengthy study related travel, blood specimen mailers and questionnaires will be sent for patients who live far from COH. Specimens will be returned by express mail.

8.0 AGENT INFORMATION

8.1 Triplex and placebo –information applicable to vaccine components

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

- cGMP 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 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 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 Northside and DFCI 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 Triplex

8.2.1 Description

The Triplex vaccine is a multiple-antigen recombinant MVA with genes encoding 3 major CMV proteins: UL83 (pp65), UL123 (IE1), and UL122 (IE2). All clinical batches of the Triplex vaccine have been manufactured at COH CBG, 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.

Triplex will be expanded on CEF grown in complete VP serum-free medium (Invitrogen). CEF will be inoculated into 407 T225 flasks at a seeding density of 4.9×10^4 cells/cm^2 and incubated for ~96 hours at 37°C, 95% humidity and 5% CO_2. After ~96 hours, viable cells will be determined from a single flask using trypan blue exclusion method. The remaining 400 flasks will be infected at an MOI equal to 0.02 using the Triplex Master Viral Seed Stock (MVSS), Batch# 0825-181-0001. Each flask, containing ~9.2×10^6 cells, will be infected with 1.8×10^5 pfu of MVSS. Cytopathic effect will be observed ~48 hours post-infection. ~4L per sub-batch of harvested crude cell suspension will be collected and ~280 mL of sample from each sub-batch will be collected for QC testing. Purification of Triplex from pellets produced from four sub-batches will be performed on 4 consecutive days. Following Benzonase® treatment (500U/mL), the virus suspension will be layered in 6 ultracentrifuge rotor tubes pre-filled with 15 mL of 36% sucrose. Tubes will be spun at 32,000xg using a Beckman Optima L90K for 80' at 4°C. After the second and final wash step, the effluent will be removed and viral pellets will be reconstituted in a total of 24mL of 7.5% Lactose/PBS. The clinical lot will be prepared by thawing bulk product containers at room temperature and pooling 4 purified sub-batches. The combined pooled bulk total will be ~80 mL. Prepared pooled bulk will be diluted to achieve a final concentration of $5.0\text{-}6.0 \times 10^8$ pfu/mL in 7.5% lactose/PBS.

The release testing of the vialed 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 Triplex vaccine was performed at Southern Research (Birmingham, AL; Study # 13928.01.01). The IND is held by COH (BB-IND #15792).

8.2.2 Supplier

Triplex vaccine is being produced, formulated and provided by COH CBG. COH IDS pharmacy or COH CBG will supply Northside and DFCI with Triplex vaccine for this study.

Triplex vaccine is supplied frozen at 5.1×10^8 pfu/mL or 9.1×10^8 pfu/mL in the formulation buffer of PBS containing 7.5% lactose. COH CBG has filled vials with cGMP 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).

8.2.3 Storage and stability

Triplex is to be stored in a monitored freezer between -60 to -90 °C. Stability analyses of Triplex are performed every 12 months.

8.2.4 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, non-pyrogenic solution which is the diluent used in the formulation of 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 [147]. 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[147].

8.3.3 Formulation

PBS containing 7.5% lactose will be supplied in a 1.2 mL polypropylene cryovial with a fill volume of approximately 1 mL/vial. The solution contains no bacteriostatic, 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 Northside and DFCI.

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 Triplex injection

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

Note: the Triplex vaccine must be administered within 4 hours after thaw.

Thawing and preparing Triplex injection

Before the scheduled patient administration, obtain frozen 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 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 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 injection

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 the vial contents to thaw at room temperature, then keep cold by placing vials on the cooling block, maintained at 4-8°C.
- Vortex the vial (containing approximately 1.0 mL of volume) for 30 seconds at highest setting. Centrifugation is not required.
- Withdraw 0.55-1.0 mL (depending on vaccine Lot) 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 syringe 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 placebo 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. Samples drawn for immunologic analysis will be analyzed retrospectively in batch, to improve precision of comparisons.

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 d42, d56, d70, d100, d114, d128, d140, d180, d210-240, d270 and d365 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 in Fox South at COH or at DFCI or Northside. 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. All samples from Northside and DFCI will be shipped as whole blood. 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 Fox South laboratories. 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 time point (e.g. d42), date of collection, and study protocol number (COH 19065), participant study number and site (e.g. Northside, COH or DFCI).

* 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.

9.1.3 Analytical Method

CMV-specific CD8 and CD4 T cells

All patients will undergo immunogenicity evaluations using Flow cytometry (FC). Blood samples for research are collected according to the study calendar to measure CMV-specific T cells by FC at COH. We will longitudinally evaluate the CMV-specific T cell response elicited by Triplex by measuring the levels of the CD137 surface marker expressed on $CD3^+$ $CD8^+$ and $CD3^+$ $CD4^+$ T cells stimulated for 24 hours with either pp65, IE-1-exon4 and IE2-exon5 overlapping peptide libraries ($pp65_{138}$ at 1 mg/mL; BEI Resources, NIH, Bethesda; IE1[128] and IE2[53] at 2 mg/mL; synthesized in-house). CD137 is expressed only on recently activated T cells, and its expression correlates with functional activation of T cells [148, 149]. Multifunctional, actively proliferating CMV-specific T cells with antiviral activity have a predominantly differentiated memory phenotype that is characterized by loss of membrane expression of the costimulatory molecule CD28 (TEM, effector memory T cells), and include large T cell populations which re-express the RA isoform of CD45 (TEMRA, $CD28^-CD45\ RA^+$)[150-153]. Thus, measurements of CD137 levels will be combined with immunophenotyping studies by using antibodies to CD28 and CD45RA cell surface markers as recently described to identify percentage of effectors (TEM and TEMRA), memory (TCM, $CD28^+\ CD45^-$) and naïve ($CD28^+CD45^+$) CMV-specific T cells. We will utilize a modified 6-parameter FC approach as we published for $CD4^+$ and $CD8^+$ T cells[69]. All immunophenotyping will be conducted on freshly thawed PBMC without stimulation *in vitro*. PBMC will be stained with each fluorochrome-conjugated antibody combination using standard methods (BD Biosciences, San Jose, CA), as described in

our published studies [112],[154]. In combination with the complete blood count (CBC), we will be able to calculate the absolute number of pp65 or IE1-exon 4 or IE2-exon 5–specific CD8 and CD4 T cells/L and their phenotypic profiles. The phenotypic ratios and levels of CMV-specific T cells will be related to control of CMV viremia as a 2^0 endpoint.

PD-1 expression [155] will be measured on $CD137^+$ T cells following stimulation with pp65, IE-1-exon4 and IE2-exon5 peptide libraries. Assessment of CMV-specific T cell growth kinetics will be performed using CFSE dilution, while cell death will also be evaluated using ApoAlert (Clontech) [156]. Reduction of PD-1 expression, decreased apoptosis and increased proliferation of CMV-specific T cells is expected in Triplex (VA) injected compared to placebo injected (PA) HCT-R. Based on recent mechanistic studies on the fate of T cells during chronic viral infections, the differential expression of the T-box transcription factors T-bet and Eomesodermin (Eomes) will be measured[161-163]. Combining intracellular Eomes and T-bet analysis with measurements of CD137 levels and T cell memory markers detailed above will better define if Triplex compared to placebo alters the recognition, activation and differentiation properties of expanded, resident or circulating CMV-specific T cells.

$CD57^+NKG2C^{Hi}$ NK cells

Characterization of $CD57^+NKG2C^{Hi}$ NK cells, known to play a role in CMV infection [157-160] will be performed by flow cytometric analysis after staining PBMC samples with the following antibodies: CD3, CD16, CD56, CD57, NKG2C (BD Biosciences).

9.2 MVA vector persistence

As recommended by FDA, persistence of the Triplex vaccine in the blood of vaccinated patients will monitored during the 12 month observation period after receipt of both doses of vaccine.

We developed and published a strategy of simultaneously detecting the MVA backbone and CMV transgenes for analysis of stability of the vector through passage by real-time qPCR using SYBR®-green and conventional short oligonucleotides as required by the FDA [127]. As per the FDA guidance, we will employ sensitivity of <50 copies of MVA/μg genomic DNA to the analysis utilizing longitudinal blood specimens drawn on d100 and d180 from trial participants. 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 a single batch at end of the study after un-blinding procedures have been completed.

10.0 STUDY CALENDAR

Table 3. Study Activity Calendar

	Pre-Vaccination				Vaccination and Bi-Weekly Visits [b]					Post-d180 Follow-Up [c]		
Study day [a]	** -60 to 0 (pre-HCT)	42	56	70-93	100 [d]	114	128 [d]	140	180	210-240	270	365
Informed Consent [f]	X											
Medical history & demographics [g]	X											
Concurrent medications [h]				X[i]	X	X	X	X	X	X[j]	X	X
Physical exam and vital signs [k]				X	X		X					
KPS performance status [l]				X	X		X					
Adverse event (CTCAE) assessment [m]					X	X	X	X	X	X	X	X
Engraftment status [n]					X	X	X	X	X	X	X	X
Disease relapse [o]					X	X	X	X	X	X	X	X
GVHD assessment and grading [p]					X[q]	X	X[r]	X[r]	X	X	X	X
CMV disease [s]					X[t]	X	X[t]	X	X			X[u]
CMV qPCR [v]					X	X	X	X	X	X[w]	X	X
HIV, HCV, CMV, active HBV [x]	X											
Pregnancy test [y]					X		X					
Chemistry/metabolic panel [z]					X		X					
CBC with differential [aa]		X	X	X	X	X	X	X	X	X	X	X
Research blood sample [bb]		X	X	X	X*	X	X	X	X*	X	X	X*
Criteria review				X[cc,dd]	X[ee]		X [ee]					
Registration/verify with DCC	X[dd,ff]											
Vaccine administration [gg]					X		X					

** Criteria review, screening, consent and Registration with DCC permitted d-60 to d0

a. Study day is defined relative to the day of HCT which is defined as d0.
b. Window for post-HCT d100 and Bi-Weekly Assessment procedures is the assigned day +/- 5 days.
c. Windows for the 'Post-d180 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 d142 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. Medical history and demographics– to include any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.
g. 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.
h. Concurrent medications pertaining to eligibility criteria will be reviewed.

i. 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).

j. Concurrent medication data collected after d180 is limited to anti-viral medications (including start and stop date).

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

l. KPS scale is found in Appendix C.

m. Adverse events (AEs) will be assessed and documented in the source documents and the case report forms at defined study visits and at standard 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 d100 to d180. All serious adverse events as defined in Section 7.3.4 will be reported up to d180. All AEs considered possibly, probably or definitely related to study agent will be reported from d100 until d180. See Section 7.0 for AE reporting.

n. 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).

o. 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 d365.

p. 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.

q. For the d100 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.

r. For the d128 visit, assessment and grading of GVHD between the initial vaccination and d128 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. Clinically confirmed CMV disease will be captured in the case report forms. Presentations or suspected presentations of CMV disease in the absence of qPCR ≥625 IU/mL will be evaluated by the treating investigator in conjunction with the blinded PMT before a determination is made.

t. 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.

u. For the d365 visit, the treating investigator will investigate and document whether CMV disease occurred d180 onward, and so note the findings accordingly. The case report forms will be updated to record any positive results not yet documented.

v. CMV qPCR will be performed at minimum bi-weekly between d100 and d180 post-HCT. CMV results will be collected in the case report forms.

w. Post d180, CMV qPCR to be performed only if clinically indicated or per institutional SOC. All CMV qPCR results will be collected in the case file.

x. CMV serostatus, HIV antibody, Hepatitis 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.

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

z. 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.

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

bb. 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 Fox South, Northside or DFCI Research laboratory. Approximately 27 ml and 3 ml are allocated for PBMC and plasma isolation respectively. * indicates that at d100, d180 and d365 one additional tube with approximately 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 (5 ml, EDTA) at each research blood time point until d100, for CMV harmonization measurements.

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

dd. d100 Vaccine Administration Criteria are found in Section 6.1. Participants failing to meet d100 vaccine criteria will complete the study at this time and be replaced and the study team should promptly inform the DCC.

ee. d128 Vaccine Administration Criteria are found in Section 6.2. Participants failing to meet d128 vaccine criteria will continue with remaining study procedures; participants with disease relapse will continue for survival follow-up only.

ff. 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.

gg. Vaccine administration is detailed in Section 5.0.

11.0 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

The primary aims of this trial are to estimate the efficacy of 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 endpoint:

1) Clinically significant CMV reactivation prompting antiviral therapy, or CMV disease (defined by histology) from d100 post-HCT and to d180 post-HCT. Death or relapse/progression prior to d180 post HCT will be counted as competing risk events. Patients who are alive and free of CMV events and relapse/progression at the last follow-up or d180 post HCT, whichever comes first, will be censored.

Secondary endpoints:

1) Key safety endpoints: non-relapse mortality (NRM) at d365 post-HCT, severe (grade 3-4) acute GVHD, and grade 3-4 AEs probably or definitely related to the vaccination and MVA vector persistence.
2) CMV-related events: duration of viremia, duration of anti-CMV therapy, peak CMV PCR value, recurrence of CMV viremia, incidence of late CMV reactivation or disease at d365.
3) Transplant-related events: time to engraftment, incidence of aGVHD, chronic GVHD, relapse, non-relapse mortality, all-cause mortality, infections and overall survival at d365.
4) Immunological function: levels and kinetics of CMV-specific T cell immunity, combined with immunophenotyping [34, 144, 145], and functional studies[161, 164]. NK phenotype and function (cytotoxicity and cytokine production) at d365.

12.0 STATISTICAL CONSIDERATIONS

12.1 Study Design

This is a randomized, blinded and placebo controlled Phase II trial conducted at three centers, COH, Northside and DFCI with data coordination at COH. The primary aim of the trial is to assess the property of Triplex to protect CMV-positive haploHCT recipients from CMV events. A secondary aim is to estimate the effect of Triplex on levels of CMV cellular immunity in HCT-R+ [165-167] and to describe the association of CMV cellular immunity with CMV reactivation events. Additional secondary aims are to evaluate safety and to evaluate the effect of Triplex on transplant-related outcomes and CMV related events e.g. the duration and treatment of viremia. The study includes rules for periodic safety monitoring.

12.2 Randomization

The trial has a target of 128 randomized adult HCT recipients. We estimate that 151 enrolled patients will yield 128 randomized and evaluable subjects to d180. A computer-generated randomization, stratified by donor CMV serostatus and center, will assign registered participants to the 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 'd100 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 (Triplex or placebo) will be considered "randomized", as there is no opportunity for a drop-out bias prior to injection in this double-blinded 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 (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

The primary objective is the comparison of the rate of clinically significant CMV reactivation (CMV-directed antiviral therapy, or CMV disease) in patients receiving vaccine or placebo. Event times will be defined as the first event meeting the definition. The time of CMV disease will be used in cases that are not preceded by detectable reactivation. Statistical methods will be based on time-to-event analysis with competing risks. A conservative power calculation is based on reactivation by d180 as a binary event. A reactivation rate of 27% on the PA is based on data described in Section 2.4 for subjects who have not reactivated prior to d100 post-HCT. We estimate that 151 enrolled participants will yield 128 randomized, evaluable subjects to d180, permitting a 63% reduction (from 27% to 10%) in reactivation to be detected with 80% power using a 1-sided hypothesis test at 0.05 level of significance, based on simplified comparison of binary rates with one interim analysis for futility when a half of the information has been collected and a final analysis. A single-boundary (non-binding futility) design is used. Spending functions with Rho Family (ρ=4.3) are chosen to derive futility boundary. Kaplan-Meier estimate of d100 survival is 89.8% among 119 recipients of haploHCT at COH in the past 5 years (unpublished). Assuming 15% all-cause attrition, a total of 151 randomized patients should provide at least 64 per arm that are evaluable at d180, in 8 years of accrual. Our previous work showed that recurrence was strongly influenced by donor CMV status, hence incorporation of time-to-events and recurrent events should increase power [14]. A

sample size of 128 patients (64 per arm) provides overall one-sided type I error of 5% and 80% power for a one-sided test to detect the difference of 17% in the rate of clinically significant CMV reactivation between 2 arms. Sample size and boundary calculation were obtained using EAST 6.4

12.4 Data Analysis Plan

Analysis of CMV events: The primary statistical analysis will compare vaccine and placebo regarding cumulative incidence of CMV events from first injection to d180 post HCT using Gray's test. Clinically significant CMV reactivation is defined in section 11.0.

Kaplan-Meier curves and log-rank test will be used to compare overall survival. Cumulative incidence curves and Gray's test will be used to compare aGVHD, cGVHD, infections, relapse, and NRM. Repeated measures analyses will be used for immune responses.

12.5 Safety Monitoring

Since HCT entails substantial risks, monitoring for expected AEs involves rate comparisons, and it entails some risk of false alarms. Since the number of control subjects is initially small, early safety monitoring will involve comparisons of vaccine groups to historical benchmarks for haploHCT. Safety monitoring will involve interim analyses with explicit, protocol-specified stopping rules. Because interim safety monitoring focuses on the vaccine arm, it will involve unmasking treatment assignments, and will be done by an independent statistician from the COH Biostatistics Core in conjunction with the independent external DMC. The independent statistician from the COH Biostatistics Core will have access to unblinded data. Early safety monitoring will be based on comparison of severe GVHD, secondary graft failure and treatment-related death to historical rates.

Final analysis of HCT-R safety will be based on comparison of vaccine and placebo regarding severe GVHD, immune reconstitution, and survival. Establishing equivalence of secondary graft failure and GVHD rates with and without vaccine is infeasible with modest sample sizes appropriate to a Phase 2 trial, but comparisons will be made using standard methods for right-censored data with interim analysis using standard group-sequential testing[169]. While unlikely that vaccine would have a deleterious effect on survival, and safety will be directly assessed as described above, early death removes patients from the set at risk for CMV reactivation. To avoid possibility of such an effect favoring VA, we plan a 2^0 analysis comparing event-free survival in both VA and PA. No systematic increase of grade of acute or chronic GVHD was observed in the in the VA in the HLA-matched Phase 2 trial (NCT02506933).

Stopping rules were developed using Pocock stopping boundaries [170]. We anticipate grade 2 AEs related to vaccination, and this caution will be explained in the consent form.

(1) d180 NRM will be monitored as every 20^{th} subject on the VA reaches the d180 evaluation point, i.e. d180 of follow-up or death. Operationally, the CRC will notify the monitoring statistician as cohorts of 40 patients (20 vaccinated) near the d180 mark. The 3^{rd} evaluation will be at the 64^{th} subject on the VA, and will be a final test for excessive NRM. Stopping rules are specified in Table 4, which gives the maximum tolerated d180 NRM at each monitoring point. If NRM frequencies exceed these bounds, which are based on historical rates, the study will be suspended for safety review by the external DMC. These numbers were selected to limit the overall false-alarm probability for this endpoint to 0.03 when there is no additional risk due to immunization, and the expected NRM rate in this haploHCT population may be 20%.

(2) Severe GVHD or secondary graft failure will be monitored as every 20^{th} subject on the VA reaches the d180 evaluation point, i.e. d180 of follow-up or a grade 3-4 aGVHD or severe cGVHD event. aGVHD will be scored using Keystone consensus criteria [171]. Whenever possible, aGVHD will be confirmed

histologically with microscopic review. Stopping rules for severe GVHD or secondary graft failure are also specified in Table 4. The study will be interrupted for safety review by the external DMC if 6 or more of the first 20 recipients experience severe GVHD or secondary graft failure. This would be a significant elevation from the expected maximum rate of 15% based on COH historical benchmark of 13.7% of haploHCT-R (M. Al-Malki et al, ASH 2017 Abstract). However, any smaller critical number would entail an unacceptable false alarm rate. These rules are determined to limit the overall GVHD or secondary graft failure false alarm probability at any 1 of the 3 monitoring times to 0.02 if vaccination does not increase risk [172], for a total false alarm rate of 0.031 across all three monitoring points.

(3) Event-Free Survival (EFS) will be defined as the time from HCT to severe GVHD, relapse, or death. The reason for evaluating this endpoint is to guard against the possibility that vaccination may have multiple deleterious effects. EFS combines potential adverse outcomes so that they reinforce, rather than disguise each other. Since the ad hoc nature of this endpoint comparison of the VA to concurrent controls is necessary, making the endpoint too variable for effective interim monitoring. EFS will be analyzed at the end of the study to produce a 90% 1-sided confidence limit on the hazard ratio. The EFS analysis thus provides due diligence in checking for a substantial adverse impact, although no difference in EFS is expected.

Table 4. Stopping rules for mortality and severe GVHD/secondary graft failure
(>given number of events will stop the trial)

Clinical endpoint	Number of patients treated		
Total Recipients at d180 (VA and PA arms – for identifying analysis time)	**40**	**80**	**128**
Expected # of recipients on VA (masked information)	**20**	**40**	**64**
Suspend accrual if VA recipients with NRM at d180 (20% expected)	**>6**	**>11**	**>15**
Suspend accrual if VA recipients w/severe GVHD/secondary graft failure (15% expected)	**>5**	**>9**	**>12**

(4) Simultaneously monitoring NRM, severe GVHD, and secondary graft loss safety outcomes generates an 11% chance of at least 1 false alarm if all safety outcomes were stochastically independent and at their expected and tolerable rates, with no excess risk due to vaccination. Since there is some overlap of endpoints, and some risks may be slightly lower than the tolerable rates cited, we expect the overall false alarm rate to be <10%. We believe this constitutes the most cautious safety monitoring plan that is feasible. Any crossing of the monitoring boundaries would immediately stop enrollment, and a decision by the external DMC would be required before the trial could be re-opened.

12.6 Interim analyses

One interim futility analysis will be performed after half of the patients (n=64) are evaluated for the primary endpoint. A final analysis after accrual and follow up for the primary endpoint is complete will be carried out. No efficacy interim analyses will be conducted. The stopping rules serve as a trigger for consultation with the DMC for additional review of safety endpoints and secondary endpoints, and are not formal stopping rules that would suspend accrual immediately. External DMC will monitor the study regularly.

Non-binding futility boundary (Table 5) is calculated using Rho family spending functions with ρ equal to 4.3. When 64 patients have been evaluated for the primary endpoint, we will perform interim futility analysis. The rate of CMV events will be calculated by arm. The test statistic and one-sided p value will be computed. If the rate of CMV events is higher in VA arm than PA arm (the difference >5%), Z statistic >

0.517, or one-sided p value >0.697, we would suggest DSMB to review the trial. Operating characteristics of the design is show Table 6.

Table 5. Interim non-binding stopping rule

Single boundary non-binding stopping rule with one-sided type I error 5%, Power 80%, the difference of 17% in CMV event rates at d180 between 2 arms and polled estimate for variance

Information Fraction	Sample Size	Cumulative β spent	Boundary for difference in CMV event rate (VA-PA)	Boundary for p value	Futility Boundary Z statistic	Conditional power boundary under alternative
0.5	64	0.01	**0.05**	**0.697**	**0.517**	**0.132**
1	128	0.198	**-0.113**	**0.05**	**-1.645**	

Table 6. Operating characteristics of the design

Difference in rate of CMV events between 2 arms (VA-PA)	Expected Sample Size	Overall cumulative power	Probability of Early Stopping due to futility
10%	87	0.2%	70.5%
5%	98	1.2%	50.4%
0%	109	5%	30.3%
-5%	118	16%	14.8%
-10%	124	38.7%	5.8%
-15%	127	69%	1.8%
-17%	127	80%	1.0%
-20%	128	92%	0.4%

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 7 (below).

Table 7. Data Submission Schedule

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

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 are 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, research nurse, 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/toxicity.

The PMT is recommended to meet (in person or via teleconference) to review study status. The meeting is a forum to discuss study related issues including accrual, SAE/AE/UPs 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

This is a moderate risk level study which requires automatic auditing after the first 2 research participants have been treated.)

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 integrity of 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), who are experienced in the monitoring of blinded clinical studies. Monitoring is conducted according to the COH OCTAM SOP. COH will monitor activity at sub-sites as if the study were conducted at COH.

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. Monitoring reports are provided to the site study teams, the site PI, study PI and DMC in a format that does not reveal blinded information.

15.5 Quality Assurance

Clinical site auditing 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. This trial will be audited by the City of Hope Office for Safety and Data Quality. Details of clinical site auditing are documented in the City of Hope Institutional Data and Safety Monitoring Plan (DSMP).

15.6 Risk Determination

This is a moderate risk study, as defined in the City of Hope Institutional DSMP. This determination was made because the study is an investigator initiated Phase II study.

15.7 City of Hope Data and Safety Monitoring Committee

The COH Data and Safety Monitoring Committee (DSMC) will review and monitor study progress, compliance, toxicity, safety, and accrual data from this trial via the PMT Progress Report (submitted by the Study Principal Investigator according to the frequency outlined in the City of Hope Institutional DSMP). The DSMC is composed of clinical specialists who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Protocol Management Team.

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

16.4.1 Informed Consent Form (ICF)

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.

The ICF will include all elements required by the COH-IRB and the States of California, Massachusetts, and Georgia namely: research project title, identification of investigators, purpose of the study, procedures, risks, benefits, alternatives to participation, financial responsibility, compensation, offer to answer questions, injury statement, confidentiality statement, voluntary participation and withdrawal statement, new information statement, termination of study participation without subject's consent, potential for commercial development related to research, information about the vaccine and its administration, tissue and/or fluid samples collected as part of this research, Experimental Subject's Bill of Rights, and agreement with signatures. Patient consent will be obtained for any residual research specimens being stored for future immunological testing.

Prior to submission to the IRB (initial submission and amendments), the consent and accompanying HIPAA form must be reviewed and approved by the COH DCC.

16.4.2 Consenting Procedure

Consenting will occur during the information sessions held to discuss the HCT procedure. Informed consent will be obtained by physicians only. Hematology physicians assigned to the patient will be approved by the IRB to assist study PIs in obtaining consent. The study must be fully explained, including all treatment options and potential risks. The prospective participant must be given sufficient time for deliberation, and all questions answered. Written informed consent will be obtained from either the patient or their 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. A copy of the signed informed consent and the Experimental Subject's Bill of Rights 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. Once the ICF has been signed, a physical exam and screening blood draw will be obtained. Per FDA requirements, the enrollment of the patient will be recorded in the medical record.

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 to anyone regardless of gender or ethnicity. COH, DFCI and Northside are committed to developing approaches to increase accrual of patients from under-served minority populations, especially Hispanic and African-American, into clinical research. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue and randomize approximately 128 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 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. There is a separate clinical trial exploring Triplex in children (NCT03354728).

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 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, and 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.

Participants will be known to their attending physician, other hematology/BMT staff, and to nursing staff for necessary clinical care and intervention. These persons also have had HIPAA-related training and routinely practice methods to protect PHI data. Participants' names will be assigned a study number by the Nurse Coordinator, which will be recorded on a separate log. Study number will be exclusively used to identify data collection forms, and blood specimens. No other identifier will be recorded on any of these sheets. The log with identification data and study number will be kept in a locked cabinet, in the PI's office at COH. Site-specific data will also be kept on the Cancer Center server at DFCI (password protected and backed up daily), and locked in a secure location in the BMT research office at Northside hospital. Only the principal investigator, co-investigators, research nurse, and data management coordinator will have access to trial data.

The randomization log devised by J. Palmer and maintained by the Data Quality Monitor and Clinical Trials Administrator (Department of Hematology) in a secure shared folder with access solely by the designated parties.

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 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 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. Published results from the study will be exclusively in the form of summary description of groups with no identifiable patient information included.

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

- 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 #19065- 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 19065: A PHASE II RANDOMIZED, PLACEBO-CONTROLLED TRIAL TO EVALUATE THE PROTECTIVE FUNCTION OF AN OPTIMIZED DOSE OF TRIPLEX VACCINE IN RECIPIENTS OF AN HAPLOIDENTICAL 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:
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___ Caucasian
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Diagnosis: _____</td></tr>
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