City of Hope

TITLE: CMV-MVA TRIPLEX VACCINATION OF STEM CELL DONORS TO ENHANCE CMV SPECIFIC IMMUNITY AND PREVENT CMV VIREMIA IN RECIPIENTS AFTER STEM CELL TRANSPLANT

PROTOCOL NUMBER: #18007 **PROTOCOL DATE: 07/19/2022**

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TYPE (*e.g., Pilot, Phase I, etc.*)**:** Phase I, Therapeutic

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City of Hope

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PROTOCOL NUMBER: #18007 **VERSION:** 15

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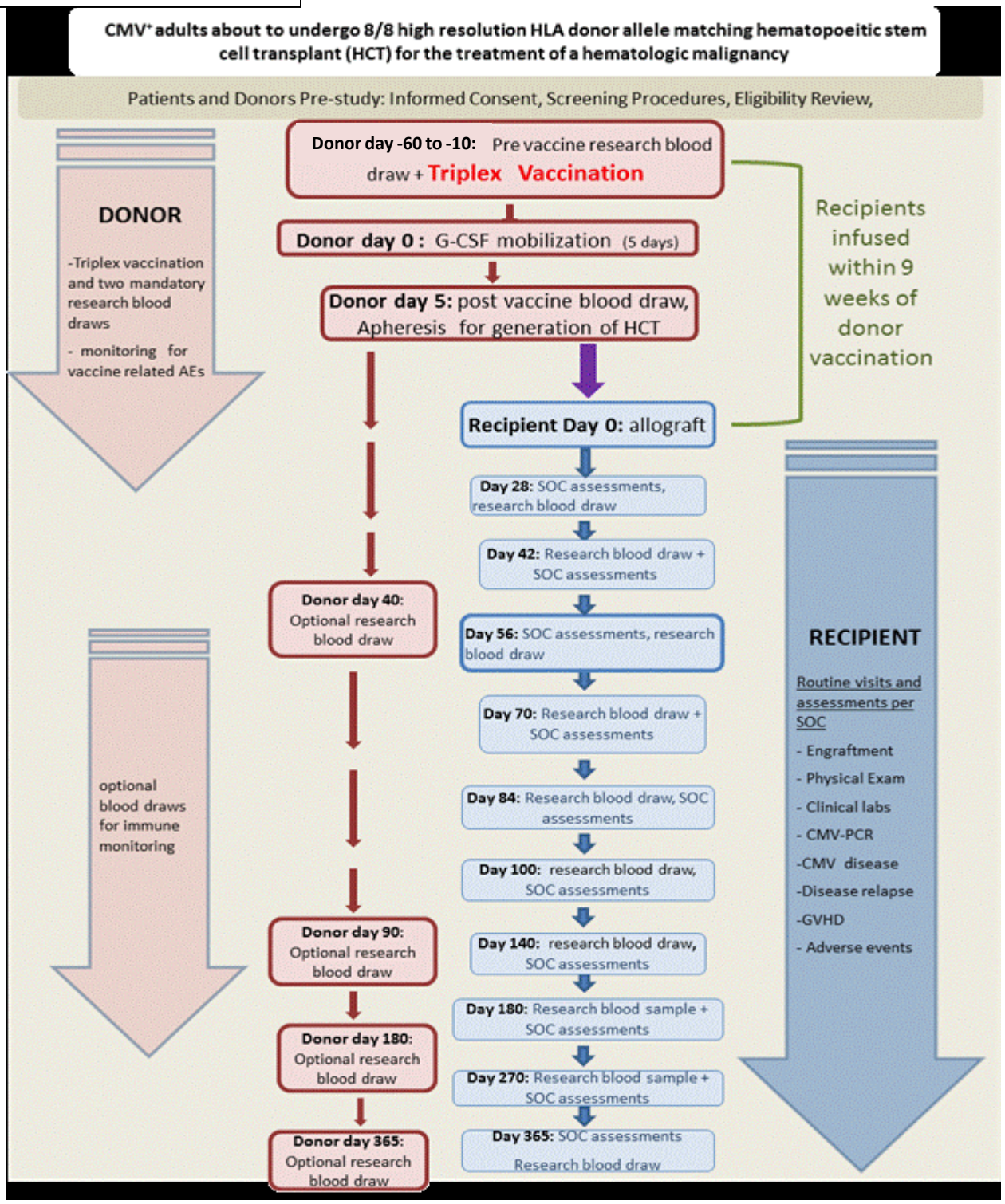
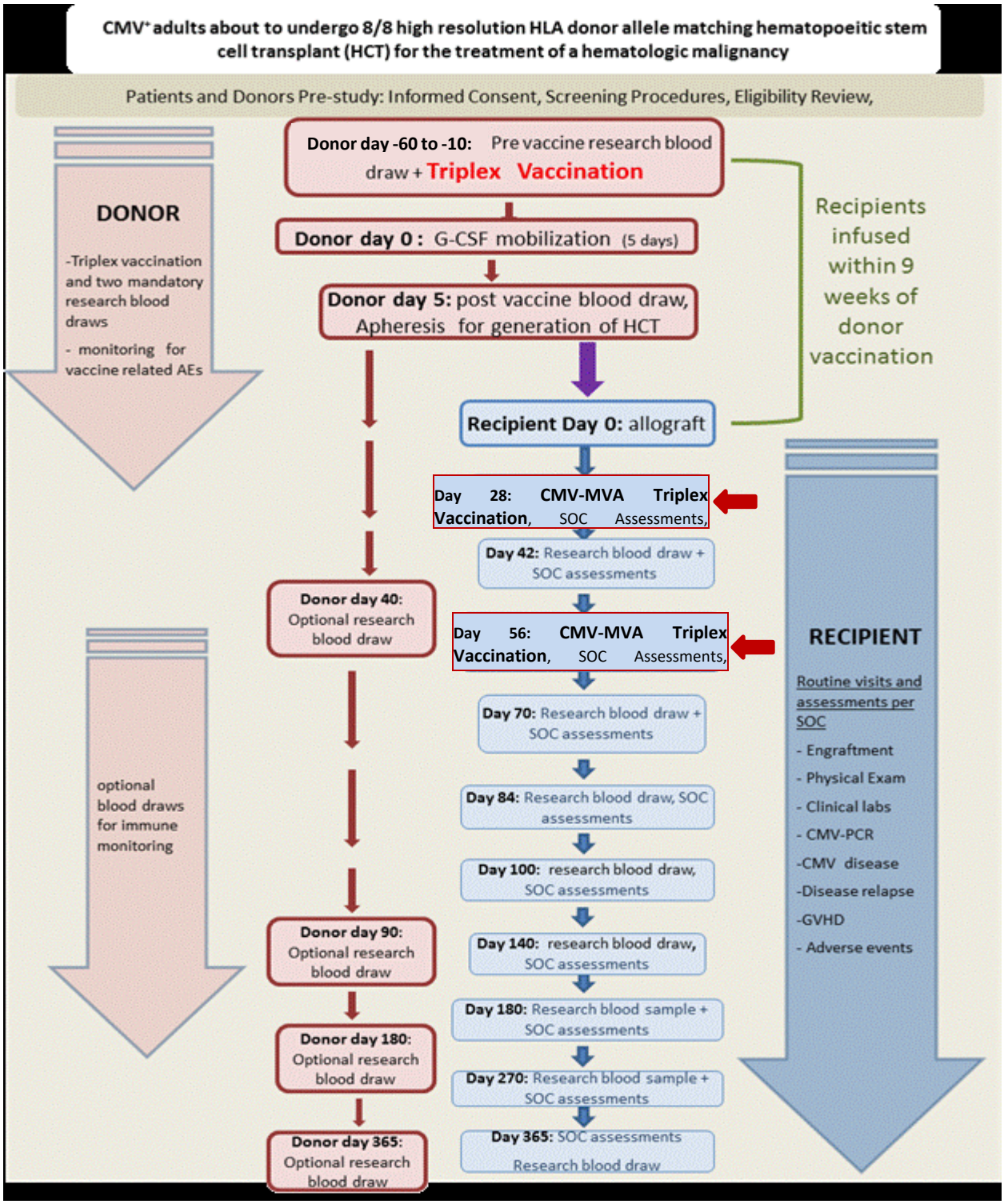
VACCINATION SCHEMA

CMV+ adults about to undergo 8/8 high resolution HLA donor allele matching or 3/6 HLA donor allele (haploidentical) matching hematopoietic stem cell transplant (HCT) for the treatment of hematologic malignancy. The arms will be recruited sequentially.

Arm 1: Donor-only vaccination



Arm 2: Donor-and-recipient vaccination



PROTOCOL SYNOPSIS

Protocol Title:
CMV-MVA TRIPLEX VACCINATION OF STEM CELL DONORS TO ENHANCE CMV SPECIFIC IMMUNITY AND PREVENT CMV VIREMIA IN RECIPIENTS AFTER STEM CELL TRANSPLANT
Brief Protocol Title for the Lay Public (if applicable):
Triplex vaccination to prevent cytomegalovirus infection after stem cell transplantation
Sponsor, IND
COH Triplex IND#15792
Study Phase:
Phase I
Participating Site:
City of Hope National Medical Center
Rationale for this Study:
Preemptive use of antiviral drugs to control cytomegalovirus (CMV) viremia in hematopoietic cell transplant (HCT) recipients can limit disease progression, but has significant side effects, including marrow suppression and neutropenia. COH has developed a vaccine based on modified vaccinia Ankara (MVA) virus, called Triplex which expresses CMV-pp65, IE1-exon4, and IE2-exon5. COH completed a safety study in healthy volunteers (HV) who were equivalent health-wise to immunocompetent HCT donors. These studies preceded a blinded Phase 2 trial that is testing clinical correlates of vaccine stimulated protection in which CMV-positive (P) HCT recipients have been randomized equally to either receive vaccine or placebo. Vaccinating recipients is innovative but carries risk that the complex antigen processing required for vaccine function will be deficient early post-HCT. We propose a small Phase 1 trial at COH. We will evaluate the safety and feasibility of vaccinating HCT donors with the Triplex vaccine to boost levels of CMV-specific T cells prior to apheresis to collect PBMC for HCT and whether Triplex vaccination of HCT recipients, in combination with donor vaccination, can further boost levels of CMV-specific T cells received from their donors and generate CMV protective immunity. The hypotheses are: 1) *in vivo* primed CMV-specific T cells from donors will expand and remain active when infused into CMV-P recipients; 2) vaccinating recipients in addition to donor vaccination will provide additional boost to CMV immunity.
Objectives:
Primary objectives: establish the feasibility and safety of priming CMV immunity in donors by Triplex vaccination prior to PBSC harvest. The hypothesis is that it is feasible and safe to vaccinate HCT donors with the Triplex vaccine and that vaccination increases the frequency of CMV-specific T cells in both donors and recipients. **Secondary objectives:** examine if Triplex vaccination of HCT donors has an impact on CMV events. Clinical observations will include the measurement of CMV viral load and the use of antivirals.

Recipients who reactivate CMV (>1250 IU/mL) and are given antiviral therapy will be considered treatment failures.

Study Design:

Overall design: This is a Phase 1 trial to evaluate the feasibility, safety and immunogenicity of Triplex vaccination in HCT donors with or without recipient vaccination. The target accrual is 18 vaccinated and evaluable donor/recipient pairs for each arm (36 donor-recipient pairs, including 72 individuals total).

Enrollment: Eligible HCT-donors (HCT-D) and HCT-Recipients (HCT-R) will be consented and enrolled pre-HCT until the target accrual is reached. HCT-D will receive Triplex injection at least 10 days prior to start of stem cell mobilization. Enrolled recipients will undergo HCT within 9 weeks of donor vaccination.

Schedule of Procedures:

Donor: Day -60 to -10: Triplex vaccination

Day -5 to -1: GCSF mobilization of vaccinated donor

Day -1 to 0: PBMC harvest and preparation of HCT graft

Recipient: Day 0*:* HCT PBSC transplant

Day 28: Triplex Vaccination (Arm 2: Donor-and-recipient vaccination arm only)

Day 56: Triplex Vaccination (Arm 2: Donor-and-recipient vaccination arm only)

NB. Donor Days are measured from the first day of G-CSF administration. Recipient days are measured from the day of transplant.

After the first three donors have received the Triplex vaccine, enrollment of further donor/recipient pairs will be paused until all three recipients reach day 42. If no SAEs attributed to donor vaccination (e.g. graft failure or early death) are observed in this first cohort, enrollment will resume.

Duration of participation: Study procedures will be completed on Day 365 post HCT for all patients who receive PBSCH from a vaccinated donor. Vaccinated donors will be followed immunologically for up to 12 months where feasible.

Endpoints:

Primary endpoints:

Feasibility: a record will be made of the number of suitable donor/recipient pairs were approached, and the number that were successfully enrolled/vaccinated.

Safety endpoints: AEs ≥ grade 2, probably or definitely related to vaccination will be noted in donors. Non-relapse mortality (NRM) at 100 days post HCT, delayed engraftment, severe aGVHD and grade 3-4 AEs (CTCAE v.4.0) probably or definitely related to the intervention will be assessed in recipients.

Therapy induced quantitative/kinetic changes in CMV-specific cellular immunity: T cells specific for pp65 and/or IE antigens will be measured as a possible correlate to protective function. The frequency of T cells specific for pp65 and/or IE antigens will be measured using CD137 expression assays. These CMV-specific T cells will be further characterized by CD107-associated degranulation, polyfunctional cytokines and cell-surface memory markers.

Secondary endpoints:

CMV protection: incidence of viremia (≥1250 IU/mL), CMV viral load and use of antivirals (recipients who reactivate CMV *and* are given antiviral therapy will be considered intervention failures).

Sample Size:

Eighteen transplant recipient/donor pairs will be enrolled for each arm. 36 donors and 18 recipients will be vaccinated total (18 donors in Arm 1 and 18 donors plus 18 recipients in Arm 2), 36 pairs with 72 donors and recipients total.

Estimated Duration of the Study

We anticipate about 100 recipient/donor pairs to be eligible annually at COH. Accrual should be completed in 4 years, and we anticipate 1 year of follow up and data analysis.

Summary of **Donor** Eligibility Criteria:

Inclusion Criteria for Donors

- Age 18 to 75 years old
- Ability to comprehend the investigational nature of the study and provide informed consent
- Willing to receive Triplex vaccination, a minimum of 10 days prior to the start of GSF mobilization

Inclusion Criteria for day 28 Donor Vaccination

- Donors are eligible to be vaccinated prior to the determination of their HIV, HBV, HCV and HTLV status. The exclusion criteria for transplant is independent of eligibility for vaccination and is determined by 3.3 Exclusion Criteria for Transplant from Donors.

Exclusion Criteria for Transplant from Donors

- Unfit to undergo standard stem cell mobilization and apheresis e.g abnormal blood counts, history of stroke, uncontrolled hypertension
- Sickling hemaglobinopathy including HbSS, HbAS, HbSC
- positive for HIV, active hepatitis B (HBV), hepatitis C (HCV) or human T-cell lymphotropic virus (HTLV-I/II)
- Donors with impaired cardiac function are excluded. Electrocardiography is routine for potential HCT donors over 60 years old and those with a history of heart disease. Subjects in whom cardiac function is abnormal (excluding 1st degree branch block, sinus brachycardia, sinus tachycardia or non-specific T wave changes) are ineligible for Triplex vaccination.
- Severe psychiatric illness sufficiently severe as to make compliance with the donation procedure unlikely, and making informed consent impossible

Summary of **Recipient** Eligibility Criteria:

Inclusion Criteria

- Age 18 to 75 years.
- Planned HCT for the treatment of hematologic malignancy with 8/8 (A,B,C,DRB1) high resolution HLA donor allele matching - low risk
- Planned HCT for the treatment of hematologic malignancy with 3/6 HLA donor allele matching (haploidentical) – high risk
- CMV seropositive
- Seronegative for HIV, HCV and active HBV
- eligible, related HCT donor willing to be vaccinated with Triplex prior to mobilization and PBSC harvest

Exclusion Criteria

- Prior investigational CMV vaccine, Experimental anti-CMV chemotherapy in the last 6 months
- No planned use of the following after HCT: Live attenuated vaccines, medically indicated subunit or killed vaccines, alemtuzumab or any equivalent in vivo T cell depleting agent, medications with known activity against CMV, CMV immunoglobulin. Letermovir allowed EXCEPT for low risk patients with 8/8 high resolution HLA donor allele matching HCT.
- Active autoimmune disease requiring systemic immunosuppressive therapy in the last 5 years
- Pregnant/nursing women

Investigational Product Dosage and Administration:

Triplex: donors in both arms will receive one dose of approximately 5 x 10^8 pfu of vaccine, administered intramuscularly. Recipients in arm 2 will receive 2 doses of approximately 5 x 10^8 pfu of vaccine, administered intramuscularly.

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 diagnostics for CMV disease assessment, according to institutional standard of care. All of the study endpoints listed above will be monitored and recorded. 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 immune phenotyping studies. Immunologic analysis will primarily be conducted on recipient PBMC, and on donor PBMC when available. The phenotypic ratios of CMV-specific T cells will be related to improved control of CMV viremia.

Statistical Considerations:

Safety:

The COH DSMC will be the DSMC of record. Safety data will be monitored as it accumulates, and donor vaccination will be suspended for DSMC review if there is evidence of serious treatment-related AEs in recipients. Specifically:

(1) Donors will be observed on the day of vaccination, and at apheresis. Any AEs attributed to Triplex vaccination will be reported to the DSMC. In a study of Triplex vaccination in healthy volunteers, vaccination was well tolerated, and the same is expected in HCT-D who are equivalent health wise. However, HCT-D will also be mobilized with GSCF for 5 days after

vaccination. The potential risk of combined toxicity from vaccination and GSCF are considered small, but must be explored.

(2) 100 days non-relapse mortality (NRM).

(3) Severe acute GVHD (aGVHD, grade 3-4) will be monitored on a per patient basis. The trial will be interrupted if 2 or more of the first 3 or 4 recipients, or if any 3 recipients experience Grade 3-4 aGVHD. This would be a significant elevation from the COH historical benchmark of 15% of allogeneic HCT recipients with matched sibling donors [1].

(4) Serious AEs (SAE, grade 3-4) probably or possibly related to the experimental therapy will be individually reviewed by the protocol monitoring team, and reported to the DSMC.

(5) Delayed engraftment will be monitored

(6) Donors will be observed on the day of vaccination and at apheresis.

Feasibility: the number of donors vaccinated as a percentage of the total donor-recipient pairs enrolled (i.e. a consenting recipient) will be calculated. With 18 donor/recipient pairs for each arm, the standard error of the percentage at most will be 0.12 with a margin of error of 0.24.

Cellular immunity: the frequency of Triplex expanded CMV specific T cells in donors will be assessed by comparing pre-vaccine and pre-apheresis blood draws. The previous study of Triplex vaccination in healthy volunteers (COH IRB#08173) showed a notable expansion of CMV specific T cells after one vaccination. HCT donors are equivalent health wise to the volunteers on this study, hence are expected to respond immunologically.

The frequency of CMV specific T cells in donors after apheresis will be assessed from day 28 onwards, until day 365 where possible. The data analysis for estimating the effect of donor vaccination on functional cellular immunity through time will be exploratory in nature, and will focus on graphical display and summary statistics. T cells specific for pp65 and/or IE antigens will be measured as a possible correlate to protective function.

Clinical Efficacy (CMV Events): Each recipient will be followed for the occurrence of CMV reactivation events, including CMV viremia, the use of CMV-directed antiviral therapy, or detection of CMV disease. Both initial and recurrent events will be recorded, with patients considered at risk for recurrent events after completion of a full planned course of anti-viral therapy. The total days on antivirals for CMV reactivation (induction, maintenance, and total) will be assessed for each individual. Evidence of clinical efficacy will be assessed within the sample size limitations of a pilot study. The target of 18 donor/recipient pairs, gives an 80% power (0.10 significance) to detect an improvement in CMV reactivation rate from 30% to 10%. Two or fewer reactivations would indicate a positive effect of the intervention (relative to the 30% benchmark) at the 0.06 significance level. Patients receiving HCT from vaccinated donors can be compared to historical controls of patients receiving HCT from unvaccinated donors. This may provide a preliminary estimate of the value of vaccinating HCT-D, with regard to protection from reactivation.

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ABBREVIATIONS

AE SAE	Adverse Event Serious Adverse Event
GVHD aGVHD cGVHD	Graft Versus Host Disease Acute GVHD Chronic GVHD
BDP	Biopharmaceutical Development Program NCI-Frederick, MD
CBC	Complete Blood Count
CLIA	Clinical Laboratory Improvement Amendment
CMV CMV-P CMV-N	Cytomegalovirus Cytomegalovirus Positive Cytomegalovirus Negative
CMV-MVA Triplex	Cytomegalovirus-Modified Vaccinia Ankara encoding 3 CMV proteins
COH	City of Hope
CRA	Clinical Research Associate/Coordinator
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTL	Cytotoxic T lymphocytes
DET	Department of Experimental Therapeutics
DSMC	Data Safety Monitoring Committee
DTM	Department of Transfusion Medicine
EKG	Electrocardiogram
FDA	Food and Drug Administration
FOS	Foscarnet
GCP, GMP	Good Clinical Practice, Good manufacturing Practice
GCV	Ganciclovir
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
ICS	Intra cellular staining
IDS	Investigational Drug Service
ICF	Informed Consent Form
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
MRD	Matched Related Donor
MVA	Modified Vaccinia Ankara
NRM	Non-relapse mortality
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
pfu	Plaque forming unit
PI	Principal Investigator
SAIC	Science Applications International Corporation
SIRS	Systemic Inflammatory Response Syndrome
HCT	Hematopoietic Stem Cell Transplant
SOC	Standard Of Care
VAL	Valganciclovir

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

Primary objectives: establish the feasibility and safety of priming CMV immunity in donors by Triplex vaccination prior to PBSC harvest. The hypothesis is that it is feasible and safe to vaccinate HCT donors with the Triplex vaccine and that vaccination increases the frequency of CMV-specific T cells in both donors and recipients.

Secondary objectives: examine if Triplex vaccination of HCT donors has an impact on CMV events. Clinical observations will include the measurement of CMV viral load and the use of antivirals. Recipients who reactivate CMV (>1250 IU/mL) and are given antiviral therapy will be considered treatment failures.

2.0 BACKGROUND

2.1 Human Cytomegalovirus (HCMV)

2.1.1 CMV and HCT

CMV is among the largest and most complex of known viruses, with a genome encoding around 165 genes [2]. Human CMV is a double-stranded DNA β-herpes virus with a high global prevalence, which rarely elicits disease in healthy immunocompetent hosts. After primary infection, CMV persists under control of cell-mediated immune (CMI) surveillance [3-6]. Although CMV-specific T cells do not eliminate the virus or preclude transmission, they can control viral replication and prevent disease. The main target for HLA Class 1-restricted T cells in healthy individuals and patients is the tegument protein UL83 (pp65)[7-9] and immediate early proteins UL123 (IE1) and UL122 (IE2) [2, 10-13].

Suppression of host immunity can elicit CMV reactivation, leading to uncontrolled viral replication and life-threatening organ damage (CMV disease) [14-16]. CMV infection causes significant morbidity in HCT recipients at early and late time points after HCT [17-19]. HCT patients are vulnerable to CMV due to immunosuppressive treatments aimed at preventing graft rejection or GVHD [20-22]. Despite prophylaxis with agents such as GCV and foscarnet (FOS), which limit virus replication [17, 23], CMV contributes to significant mortality [24, 25]. Furthermore, anti-viral chemotherapy has major side effects, including delayed immune reconstitution [18, 26] which increases the incidence of fatal infections [18, 26]. The antiviral, letermovir, has positive Phase 3 results at 100D (days) post-HCT, but protective immunity is essential to control virus long term [27, 28]. Furthermore, there are reports of letermovir resistance occurring after in vitro exposure to CMV cultures suggesting a low viral genetic barrier [29] [30]. Indeed, reactivation and disease can occur when antivirals are stopped (MK-8228-001 trial results [31]) or when virus resistance occurs [20, 32-35]. When antivirals are stopped or when anti-viral resistance occurs, similar disease symptoms appear, occurring approximately 180 days post-HCT [19, 36-38]. Thus antiviral usage does not prevent late-onset CMV disease, which includes CMV reactivation and failure to reconstitute CMV-specific immunity [24]. CMV immunotherapy that confers protective immunity early post-transplant, until normal immunocompetence is re-established, may reduce CMV morbidity and the need for toxic antivirals [39]. Early phase clinical studies in healthy CMV-negatives have shown that pp65, IE1 and IE2 antigens expressed from MVA can stimulate primary immunity to comparable levels as CMV-positive individuals [40-42], supporting the use of these antigens in the development of CMV therapeutics.

2.1.2 CMV protection in HCT Recipients

CMV interferes with immune function [42-49], altering the host response to CMV. Subunit Ag vaccination strategies aim to overcome CMV-mediated immunosuppression by limiting viremia, an independent predictor of disease [50]. Several reports have associated protection from CMV disease in HCT recipients with CMV-specific $CD8^+$T cell levels of 7-10/µL [34, 43, 45]. Studies conducted at COH demonstrated that the $CD8^+$T cell expansions can occur prior to d40 post-HCT, and can be donor derived [46]. In patients who have measurable CMV viremia, expansion of $CD8^+$ T cells occurs [45, 47] and CMV disease occurs only in patients with low levels of CMV-specific$CD8^+$ T cells [25, 44, 48]. Even CMV-negative HCT recipients show superior CMV immune reconstitution after HCT from CMV-positive donors [43, 49]. Cellular immunity in HCT recipients is augmented during CMV reactivation as shown by increased levels of functional CMV-specific $CD8^+$T cells [25, 44, 45]. Dr Diamond's group and others have demonstrated that a CMV-positive donor enhances CMV immunity in the recipientand decreases severity and duration of CMV infection [50-54]. The greatest risk of CMV viremia is a CMV-P recipient with a CMV-N donor [55], hence there is a need to improve viral competence in these patients.

2.2 HCT Allografts

Allogeneic stem cell transplantation has a high rate of cure for a variety of hematologic malignancies [56]. Data from the Center for International Bone Marrow Transplant Research (http://www.cibmtr.org/) on over 30,000 transplants performed worldwide over the last 30 years shows that relapse rates have remained unchanged (20% for early disease, 40% for intermediate disease, and 60% for advanced disease subjects). Disease-free survival follows the same trend (65% for early disease, 40% for intermediate disease, and 20% for advanced leukemia). To improve the chance of successful HCT, recipients receive chemo-radiotherapy conditioning regimen which is as intensive as they can tolerate. Success in allo BMT for hematologic malignancies requires effective graft-versus-tumor (GVT), the goal being to prevent relapse. This requires accepting some GVHD, given its tight link to GVT, and at times utilizing intensive chemotherapy during conditioning to improve disease control.

2.2.1 Complications of Allogeneic Stem Cell Transplantation

Graft-versus-host disease: *GVHD* represents a major complication of allogeneic HCT, leading to significant transplantation associated morbidity and mortality. Despite prophylaxis with cyclosporine (CSA) or methotrexate (MTX), acute GVHD occurs in 30% to 50% of subjects receiving transplants from HLA-identical siblings. Long term survival in subjects developing severe acute GVHD has generally been less than 30% [57-61]. Becuase of the risk of GVHD, it is necessary to give immunosuppression post HCT.

Infection: following allo HCT subjects are at increased risk of bacterial fungal and viral infections. Pre-emptive antiviral agents, antibiotics and effective new antifungal agents have greatly improved the management of infection after transplant. Infectious death now mainly occurs when infection complicates GVHD in the setting of intense immunosuppression.

2.3 Vaccination against CMV in HCT Recipients

2.3.1 Vaccine Strategies for CMV:

Live viral vaccination aims to induce helper and cytotoxic immunity and hence a durable memory response [62, 63]. Plotkin *et al*. developed a therapeutic vaccine, the attenuated Towne strain, in the 1970's.

However concerns regarding live CMV have minimized its applicability [64, 65]. Latter attempts include ALVAC expressing gB (UL55),which failed to elicit significant antibody levels in CMV-negatives [66, 67], and ALVAC-UL83 which stimulated robust cellular immunity in CMV-negatives equivalent to natural CMV-positives [40]. Further studies with ALVAC-UL55 and purified soluble UL55 protein demonstrated minimal efficacy [68, 69]. AlphaVax^{TM} expressing UL83, UL123 and UL55 was promising in healthy adults [41], but is unsuitable for HCT recipients since it can propagate in humans [41]. Despite priomising animal data, TransVax^{TM}, a DNA vaccine expressing either UL55 or UL83 induced only weak responses in humans [70, 71].

CMVPepVax, derived from the CMV-UL83 antigen, was safe and elicited vaccine driven immune responses when tested in healthy adults (NCT00722839)[72]. Subsequently CMVPepVax was found to be safe in HCT recipients (NCT01588015) when injected on day 28 and day 56 post-HCT, with reduced CMV reactivation and no increase in acute GVHD [73]. However, the application of CMVpepVax is restricted to the HLA A*0201 population, who comprise only ~30-40% of the HCT population. The Triplex vaccine being investigated in this protocol has no HLA restriction. It shows greater immunogenicity than DNA vaccines, and since it expresses whole CMV proteins, has broader recognition and greater applicability for HCT recipients than CMVPepVax.

2.3.2 Modified Vaccinia Ankara (MVA) as a Vaccine:

Attenuated, recombinant MVA is being evaluated as a vaccine for infectious disease and cancer, having demonstratedsafety as a smallpox vaccine [74]. Although MVA DNA efficiently replicates in mammalian cells, it is avirulent due to the loss of two host range genes during 570 serial passages through chicken embryo fibroblasts (CEF) [75, 76]. Despite restricted host range and inability to produce infectious progeny in human cells, both early and late transcription are unimpaired [77-80]. Unlike other attenuated poxviruses, the block in viral assembly does not impair production of large quantities of recombinant proteins in otherwise non-permissive hosts [81]. Multiple sites of foreign gene integration in MVA allow the virus to be modifed to express multiple full-length antigens. Despite pre-existing anti-vaccinia virus immunity and potent immune responses against the vector, repeated administration of recombinant MVA can boost responses to transgene encoded products [82-84].

Animal Studies: Studies in rodent and macaque challenge models affirm MVA's safety and its ability to confer protection against virulent forms of poxviruses in [85, 86]. MVA-based vaccines have either elicited systemic immunity or protection against influenza [87, 88], parainfluenza [89], RSV [90], dengue [91], Japanese encephalitis [92], malaria [93] and HIV/SIV [94-98] in animal disease models. Recently COH demonstrated that an MVA vaccine containing rhesus-CMV components successfully protected CMV-negative macaques from infection [99, 100].

Human Studies: After inoculation MVA remains avirulent, even under immunosuppressive conditions [80]. Therapeutic vaccination with MVA expressing HIV-1 nef demonstrated safety in HIV-1 infected individuals [101]. MVA immunization of human subjects confirmed vector safety and partial protection against a heterologous malaria strain [102, 103]. MVA expressing human MUC1 was found to be safe and elicited T cell immunity in cancer patients [104]. An MVA expressing human p53 (p53MVA) developed at City of Hope [105] has been evaluated in a Phase 1 trial in advanced gastrointestinal cancer patients (NCT01191684)[106], in Triple negative breast cancer (NCT02432963)[107] and in ovarian cancer patients (NCT02275039)[108]. Data from a randomized, placebo-controlled, double-blind study [Clinical Trials Registration: NCT00565929] showed that MVA was safe, well tolerated and immunogenic in HCT recipients [109]. No current CMV vaccine strategy using a recombinant vector incorporating multiple CMV cellular response antigens is being developed for HCT recipients.

2.3.3 Construction, Expression and Function of 3-antigen MVA Vaccine (Triplex):

Choice of antigens: Since they are targets for cell mediated immune responses, UL83, UL122 and UL123 have been selected as vaccine antigens, [11, 110-112]. UL83 is the most immunogenic CMV structural protein [7-9, 113-116], although UL123 may be comparable [2, 11-13, 117]. All three are immunodominant, and combined recognition should occur in over 95% of the population [110]. An association of cellular immunity to UL83 and UL123 with recovery from CMV-retinitis in AIDS patients has been reported [118]. Furthermore, T cells specific for these antigens accumulate in individuals with CMV reactivation episodes [119]. Although there is a strong humoral response to Triplex, there is no evidence that this neutralizes CMV [120]. The majority of the CMV-neutralizing antibody response has been localized to the gB (UL55) and UL128 gene products [99, 121-124]. Evidence that a humoral response protects HCT recipients against CMV is lacking, hence gB has been omitted from this vaccine. The Triplex vaccine focuses on the cell mediated response associated with disease protection in HCT recipients.

Functional modification of CMV genes incorporated into MVA: the regulatory activity of the UL123 protein includes trans-activating properties on various cellular promoters [125-127]. Consequently 85 aa comprising coding exons 2 and 3 have been deleted. Deletion of the two coding exons results in a cytoplasmic, 406-aa protein [11] with minimal transactivation activity [126, 127]. Most known CTL epitopes from UL123 are found in exon 4, including the HLA A*0201-restricted CTL epitopes [12, 13, 117, 128]. Exon5 of UL122 was fused in frame to exon4 of UL123 without modification [129].

Host cells for Triplex generation: MVA was derived by serial transfer (570 passages) of the parental Ankara strain through chicken embryo CEF to derive a safe alternative to the smallpox vaccine[74]. Its adaptation to CEF resulted in several genomic deletions [75, 76, 129]. These adaptations allow MVA tofreely propagate in CEF to titers exceeding 10e10 pfu/mL, whereas standard mammalian cell lines such as CV-1 are non-permissive for propagation. For the pre-clinical studies conducted under GLP, specific pathogen-free CEF, from Charles River-SPAFAS were used. Triplex vector was constructed using the pZWIIA plasmid and insertion of foreign genes by homologous recombination [75]. The modified H5 (mH5) promoter ensures sufficient protein expressionfor manufacture of a stable virus [130], providing a powerful boost to transgene expression without causing genomic instability [81, 131] (see Investigator's Brochure for vector map and further details).

2.3.4 Preclinical Evaluation of Triplex vaccine

Evaluation of the immunogenicity of the Triplex in mouse models using intracellular cytokine secretion (ICS) methods were used to evaluate both CD4 and CD8 T cells responses to the vaccine. Since Triplex activity is not HLA restricted, the vaccine was tested in various transgenic HLA mouse strains including A2, B7, A1 and A11 [129, 132] with a peptide library approach applicable to any HLA type. The results 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.

Triplex was evaluated *in vitro* for its ability to stimulate memory responses in PBMC from healthy adults and HCT recipients [129, 132]. Interestingly, Triplex induced strong expansions of CMV-specific CD4+ and CD8+ T cell subsets in both healthy adults and patients within 6 months of receiving HCT. The FDA (BB-IND #15792) and IRB (08173) granted allowance to proceed with a Phase Ib trial to evaluate the safety and biological efficacy of the TRIPLEX vaccine in healthy volunteers, with or without prior immunity to CMV and vaccinia (see below).

2.3.5 Animal Toxicology

The potential for long-term persistence of vectored vaccines and resultant side-effects is a concern. The non-recombinant form of MVA has been found to be safe in over 100,00 people [74, 133, 134] including HCT recipients [109]. However, the recombinant forms cannot be assumed to have an identical safety profile. Therefore pre-clinical toxicology studies of the GMP-grade Triplex Vaccine were conducted in rabbits at the Southern Research Institute (Birmingham, AL; Study # 13928.01.01). The vaccinated rabbits showed minimal toxicity, with the Triplex vaccine being cleared from the injection site, blood and other tissues within 46 days of administration.

2.3.6 Human Studies: Phase Ib clinical trial in healthy adults (IRB 08173)

Measuring viral persistence, maximum tolerated dose (MTD) and immunogenicity of Triplex in healthy volunteers (HV) was required by the FDA prior to treatment of HCT recipients. In the Phase I trial (NCT01941056), these endpoints were evaluated in 24 HV (age: 18-60), with or without prior immunity to CMV and vaccinia. Three escalating dose levels (DL) were administered intramuscularly (DL1=10xE7; DL2=5x10E7; DL3=5x10E8 pfu/dose) in 8 subjects/DL, with a booster injection 28d later. Subjects were followed for 1 year. As of April 2015, all 24 planned HV were enrolled, vaccinated and completed 12 months of planned follow-up. All vaccinations were well-tolerated, with no SAE or DLT. Immunogenicity of the vaccine was evaluated by measuring activation of T cells harvested from vaccinees and stimulated with full-length pp65, IE-1 and IE2 overlapping peptide libraries, or quantification of CMV-specific T-cells with HLA multimers. Triplex induced robust expansion of pp65, IE1 and IE2-specific CD8 and CD4 T-cells in vaccinated CMV positives, at each DL. CMV-specific T-cells with common HLA alleles and corresponding CMV-CTL epitopes were identified. Statistical analysis indicated that post-vaccination levels of pp65-, IE1- or IE2-specific CD8 and CD4 T-cells were significantly increased (p-values ranging from 3 $x10^{-5}$ to 0.025). Importantly, robust immunity was detected in CMV negatives and in subjects who had received smallpox vaccinations. Elevated frequencies of CMV-specific CD4 and CD8 T cells for all 3 antigens plateau after day 56, and in some cases remain elevated for one year [135]. Furthermore, naïve T cells dropped during the vaccination phase and terminal effector-memory T cells rose, suggesting effective recognition by CMV-specific T cells followed by evolution to a more mature effector-memory phenotype. PCR assessment of circulating vector showed minimal residual MVA DNA [10-30 gc/mL] post-injection in 2 vaccinees in the DL3 cohort, which disappeared within 3 months. This supports the hypothesis that CMV^+ patients receiving allografts from CMV^+ or CMV^- donors would generate protective CMV-specific immunity after vaccination with Triplex.

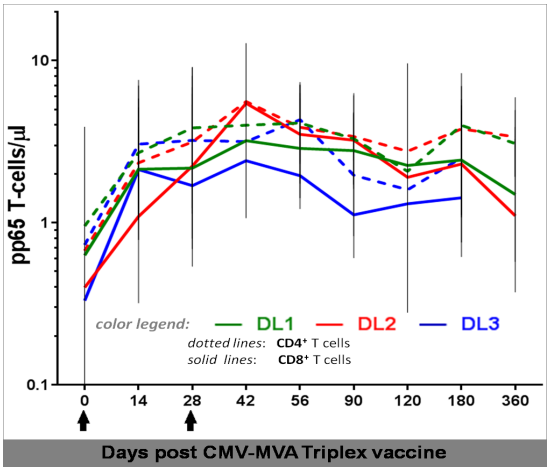


Figure 1: Measurement of T cell responses in 24 healthy research subjects pre and post vaccination (d14-d360). Using the CD137 T cell activation assay after incubation of PBMC with 138 peptides comprising the pp65 antigen (Pepmix, JPT) from Longitudinal blood draws from each of 8 vaccine recipients. Arrow =injection.

2.3.7 Triplex in Allo-HCT recipients

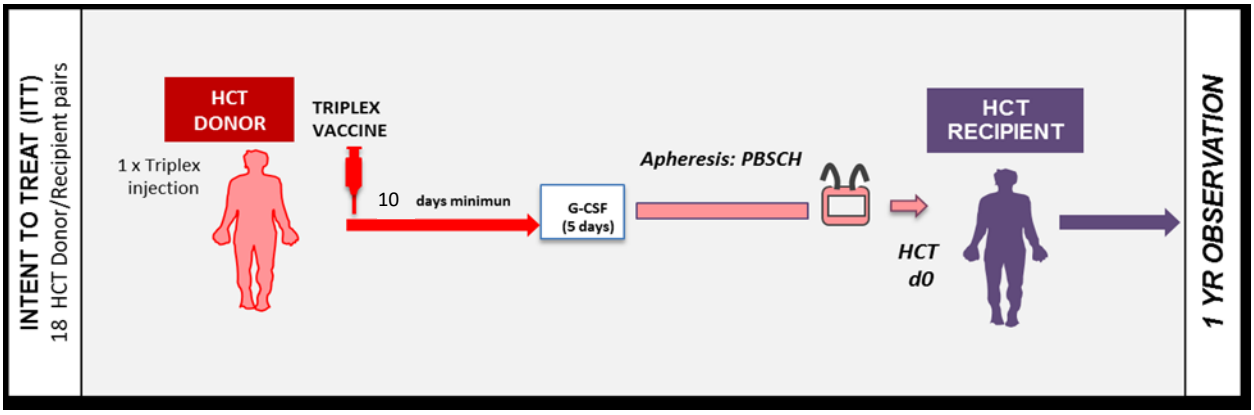
COH has completed a double blind, randomized Phase 2 trial to evaluate the efficacy of the Triplex vaccine in protecting against CMV viremia and disease in allo HCT recipients. The FDA (9/25/15) allowed our clinical trial entitled, "A Phase 2 randomized placebo-controlled trial to evaluate the protective function of a Triplex vaccine in recipients of an allogeneic hematopoietic stem cell transplant (NCT02506933)." The target accrual is 115 patients, to ensure randomization of 102 allo HCT recipients to the vaccine and placebo arm (n=51 each arm). Eligible HCT recipients are CMV-positive with an 8/8 high resolution HLA-matched donor, either sibling or unrelated. The primary objectives are safety and tolerability of Triplex according to the following: non-relapse mortality (NRM) at 100d post-HCT, severe (grade 3-4) acute (a)GVHD, and grade 3-4 AE (CTCAE 4.0) probably or definitely related to the vaccination within two weeks from each vaccination. In addition, we will determine if Triplex reduces frequencies of CMV events. CMV events encompass CMV reactivation (DNAemia ≥1250 IU/ml by qPCR), viremia treated by antivirals, or detection of CMV disease by tissue histology (end-organ disease). Secondary objectives include measurement of virema and treatment duration, HCT-related outcomes to include aGVHD, cGVHD, relapse, NRM, and opportunistic infections. This is a multisite study (COH, DFCI and MDA) with anticipated completion of accrual and 365 day assessment by October 2018. *Current Status*: accrual complete. No patient receiving 1 or 2 injections has experienced an SAE (unpublished data).

2.4 Overview and Rationale of Proposed Study

This will be an open-label study of an investigational approach to CMV prophylaxis in CMV-P HCT recipients.

Triplex vaccinated HCT donors will be mobilized with GCSF for 5 days prior to apheresis to collect PBSC for preparation allograft preparation. PBSC from the donor will be transfused into the CMV-P recipient with the aim of conferring protective anti CMV immunity. Antiviral treatment for viremia will be considered a failure of donor vaccination to provide protection to the recipient. The study has a target of 18 HCT donor/recipient pairs. After the first three donors have received the Triplex vaccine, enrollment of further donor/recipient pairs will be paused until all three recipients reach day 42. If no vaccine related SAEs have been observed in this first cohort of donors and recipients, enrollment will resume. All HCT recipients will be followed until day 365 post-HCT for safety, virologic and immunologic assessment. All donors will be monitored for vaccine induced adverse events.

Figure 2: Study Schema



PBSCH: peripheral blood stem cell harvest

3.0 PARTICIPANT ELIGIBILITY CRITERIA

3.1 Inclusion Criteria for Donors

- Age 18 to 75 years old
- Ability to comprehend the investigational nature of the study and provide informed consent
- Willing to receive Triplex vaccination, a minimum of 10 days prior to the PBSC collection

3.2 Inclusion Criteria for Donor Vaccination

- Donors are eligible to be vaccinated prior to the determination of their HIV, HBV, HCV and HTLV status. The exclusion criteria for transplant is independent of eligibility for vaccination and is determined by 3.3 Exclusion Criteria for Transplant from Donors.

3.3 Exclusion Criteria for Transplant from Donors

- Unfit to undergo standard stem cell mobilization and apheresis e.g abnormal blood counts, history of stroke, uncontrolled hypertension
- Sickling hemaglobinopathy including HbSS, HbAS, HbSC
- positive for HIV, active hepatitis B (HBV), hepatitis C (HCV) or human T-cell lymphotropic virus (HTLV-I/II). This holds true even if the donors have been already vaccinated according to criteria in 3.2
- Donors with impaired cardiac function are excluded. Electrocardiography is routine for potential HCT donors over 60 years old and those with a history of heart disease. Subjects in whom cardiac function is abnormal (excluding 1st degree branch block, sinus brachycardia, sinus tachycardia or non-specific T wave changes) are ineligible for Triplex vaccination.
- Severe psychiatric illness. Mental deficiency sufficiently severe as to make compliance with the donation procedure unlikely, and making informed consent impossible.

3.4 Pre-HCT Inclusion Criteria for Recipients

Patients 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.

<u>Age Criteria</u>

___ 3. Age 18 to 75 years.

<u>Nature of Illness and Transplant Related Criteria</u>

___ 4. Planned HCT for the treatment of the following hematologic malignancies: lymphoma (Hodgkin and Non-Hodgkin), myelodysplastic syndrome, acute lymphoblastic leukemia in first or second remission, acute myeloid leukemia in first or second remission, chronic

myelogenous leukemia (in first chronic or accelerated phase, or in second chronic phase), chronic lymphocytic leukemia, myeloproliferative disorders and myelofibrosis (COH only). Patients with multiple myeloma are excluded.

___ 5. CMV seropositive

___ 6. Planned related HCT with 8/8 (A,B,C,DRB1) high resolution HLA donor allele matching or 3/6 HLA donor allele matching (haploidentical),

___ 7. Conditioning and immunosuppressive regimens according to institutional guidelines are permitted

Clinical laboratory parameters

___ 8. Negative serum or urine β-HCG test (female patient of childbearing potential only) within two weeks of registration.

___ 9. Seronegative for HIV, HCV and active HBV (Surface Antigen Negative) within 2 months of registration.

Child Bearing Potential

___ 10. Agreement by females of childbearing potential and males with partners of childbearing potential to use effective contraception (hormonal or barrier method or abstinence) prior to study entry and for up to 90 days post-HCT. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

3.5 Pre-HCT Exclusion Criteria

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

Previous therapies

___ 1. Any prior investigational CMV vaccine

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

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

___ 3. Live attenuated vaccines

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

___ 5. Allergy treatment with antigens injections

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

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

___ 8. Prophylactic therapy with CMV immunoglobulin or prophylactic antiviral CMV treatment (Letermovir is permitted EXCEPT for low risk patients (8/8 high resolution HLA donor allele matching HCT))

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

___ 10. Other medications that might interfere with the evaluation of the investigational product (see Prohibited Medications, Section 5.5)

Other illnesses or conditions

___ 11. Diagnosis with autoimmune disease

___ 12. Pregnant women and women who are lactating. The risks of CMV-MVA-Triplex 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. Breastfeeding should be discontinued if the mother is enrolled on this study.

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

Noncompliance

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

3.6 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

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.

4.1.1 Subject Enrollment

All subjects (donors and patients) will be registered centrally on the study by the Data Coordinating Center as follows:

1. The data manager/coordinator/research nurse should contact the DCC via telephone or email

to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window (60 days to 0 days before planned HCT).

2. The data manager/coordinator/research nurse should then e-mail copies of the following documents to the DCC:
   - Completed Eligibility Criteria List
   - Source documentation to support eligibility criteria
   - Signed informed consent document
   - Signed HIPAA authorization form (if separate from the informed consent document)
3. After having received all transferred documentation, the DCC will review the documents to verify eligibility, working 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 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 City of Hope DCC (DCC@coh.org)

*Medidata Rave is a web-based, 21 CFR 11 compliant database which will consist of the study specific electronic CRFs (e-CRFs) used for capturing, managing and reporting clinical research data.

5.0 TREATMENT PROGRAM

5.1 Treatment Overview

The study will consist of the following procedures in N=18 donor/recipient pairs:

Donor: Triplex vaccination (investigational), followed by standard procedure GCSF mobilization and apheresis to obtain HCT material.

Donors will be monitored for vaccine related and GCSF side-effects.

Recipient: myeloablation and conditioning, followed by HCT with PBSCH from vaccinated donor.

Recipients may receive any clinically indicated pre-transplant myeloablative therapy according to standard indications to increase the chance of successful transplantation. The PI will decide on the details of the treatment necessary in the individual case. Donors will receive one dose of approximately 5 x 10^8 pfu of vaccine, administered intramuscularly. Recipients will undergo HCT within 9 weeks of donor vaccination.

In accordance with available data on absence of survival benefit from Prevymis (letermovir) for low risk (8/8 high resolution HLA donor allele matching HCT) CMV patients, presence of adverse events attributed to Prevymis (nausea, diarrhea, vomiting, peripheral edema, cough, headache, fatigue, abdominal pain), and unknown effect of Prevymis on immune reconstitution due to vaccine administration, Prevymis is not allowed in low risk (8/8 high resolution HLA donor allele matching HCT) patients in this protocol. Moreover, since the HCT recipients are not being vaccinated themselves, but derive the benefit of donor vaccination, they will not be likely to experience any adverse events related to vaccine administration.

Additionally, due to the positive results of trials IRB#08173 and IRB#14295 for Triplex vaccine, we expect the recipients in this trial IRB#18007 to have a comparable outcome of reduced levels of CMV reactivation comparing to Prevymis administration.

After the first three donors have received the Triplex vaccine, enrollment of further donor/recipient pairs will be paused. If no SAEs are observed in this first cohort of donors, enrollment will resume.

5.2 Assessments (recipients)

All 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 9), per institutional SOC.

5.2.1 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 C).

5.2.2 CMV monitoring

For CMV monitoring, standard qPCR clinical laboratory methods will be required to evaluate CMV viral load and possible vaccine failure at least weekly or as required by SOC until day 100.

Clinical CMV disease status will be documented at each study visit, which may include the absence or presence of suspected CMV disease. When clinically indicated and per SOC, CMV disease will be assessed and, when present, the site (upper GI, lower GI, other, specify) and method of detection in the tissue (tissue culture, pathology etc.) will be documented. Presentations or suspected presentations of CMV disease in the absence of qPCR>1250 IU/mL will be evaluated by the treating physician in conjunction with the PI before a determination is made.

5.2.3 Engraftment assessment

Engraftment will be assessed by monitoring the recipient's absolute neutrophil count. The date of engraftment is defined as the first of 3 consecutive days when the peripheral blood absolute neutrophil count is $\geq 500/mm^3$; 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 greater than 3 consecutive days 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.

5.2.4 Disease relapse

Disease relapse will be assessed (including timing of assessment) and defined 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.

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

5.2.6 Adverse event assessment (CTCAE)

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

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

5.2.7 Physical exam, vital signs, medical history 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.

5.2.8 Concomitant medications

All medications, supportive care, blood products or radiation therapy taken or administered during the trial will be documented in the subject's clinical/hospital record. Additional documentation will be made, where necessary, to support the concomitant medication data collected in the study case report forms:

- 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

5.2.9 Immunogenicity testing

Research blood draws will be collected from participants for immunogenicity and MVA persistence according to the study calendar (Section 9.0). See Section 8.0 for details of sample collection, storage and processing procedures.

5.3 Criteria for Discontinuing Study Participation

Participation may continue until one of the following criteria applies:

- donor does not receive vaccination
- Failure of HCT: peripheral blood stem cell harvest (PBSCH) cannot be obtained post donor vaccination, patient/recipient pair will continue procedures off study
- Participant withdraws from the study (donor or recipient)
- General or specific changes in the participant's condition that render them unacceptable for participation in the opinion of the treating investigator.

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

5.4 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 below:

5.4.1 Patients who do not receive HCT

Participants who do not receive HCT will discontinue any further follow-up.

5.4.2 Patients who withdraw from the study after receiving HCT

HCT recipients who withdraw from the study may continue with follow-up per the participant's agreement:

- may elect to continue to be monitored until Day 365 only, or
- may elect to withdraw completely; further follow-up or assessments will not occur.

5.4.3 All other patients

All HCT recipients who receive PBSCH from Triplex vaccinated donors, and have not withdrawn from the study, will continue follow up assessments and research blood draws as indicated in the Study Calendar (Section 9) through day 365 post HCT. Study participation will be completed on Day 365 Post-HCT.

5.4.4 Donors

All donors not receiving Triplex vaccination will discontinue any further assessments. Vaccinated donors may elect to leave the study after apheresis (day 0), or continue to provide research blood draws till day 365 (see study calendar).

5.5 Supportive Care, Other Concomitant Therapy, Prohibited Medications

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

- Medications that could interfere with Triplex generated immunity in vaccinated donors are prohibited until after apheresis. 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)
- No other investigational agents may be given to HCT-R
- 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 interfering with the positive effects of the CMV vaccine.
- Preemptive therapy with CMV immunoglobulin or antivirals (GCV/VAL, FOS, Cidofovir, CMX-001) is not allowed following HCT.

- GCV/VAL, FOS, Cidofovir,CMX-001 may be used according to institutional SOC for management of CMV viremia. In general, therapy should not commence until after CMV qPCR ≥1250 IU/mL. For preemptive therapy when qPCR<500, the study PIs are to be consulted.
- Prophylactic use of letermovir is allowed EXPECT for low risk patients (8/8 high resolution HLA donor allele matching HCT).
- 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 is allowed as standard of care to prevent HSV.
- Steroid therapy with prednisone, or equivalent, is permitted according to clinical need.

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

6.0 DATA AND SAFETY MONITORING

6.1 *Definition of Risk Level*

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. This determination was made because the study involves a COH held IND and is the first use of the Triplex vaccine in HCT donors. The COH DSMC will be the DSMC of record .

Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) is responsible for monitoring the data and safety of this study. The PMT consists of the Principal Investigator (PI), Biostatistician, Research Protocol Nurse, and Clinical Research Coordinator.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).

6.2 Adverse Event Reporting

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy [policy effective date: 05/14/14]. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

Adverse Events and Serious Adverse Events: The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events using the CTCAE version 4.0.

Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

6.3 AE and UP Definitions

6.3.1 Adverse event (AE)

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

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

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

6.3.3 Expected Adverse Event

Any event that does not meet the criteria of 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.

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

A serious adverse event is defined as any expected or unexpected adverse event 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).

6.3.5 Unanticipated problems Involving Risk to Subjects or Others

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

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

6.3.6 AE Description and Grade

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

6.3.7 AE Attribution

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

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

6.4 Routine Reporting of Adverse Events

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

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

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

All adverse events must be followed until the event is resolved, stabilized, or determined to be irreversible by the participating investigator; for ongoing adverse events that are unrelated to study agent, the follow-up period may end at 30-days post study-intervention assessment.

6.5 COH Held IND

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the Medwatch 3500A are:

- 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)]

The 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 approved City of Hope's Institutional policy [policy effective date: 05/14/14].

6.1 Deviations, SSEs and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11].

Single Subject Exception (SSE)

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11]. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

Unanticipated Problem (UP) – 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.

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy [policy effective date: 05/14/14] using iRIS.

6.2 Adverse Events - Donors

All participants will have a study telephone contact number to report any suspected AEs in between hospital visits. The donor will be contacted within the first 7 days of vaccine administration to collect any adverse reactions. Adverse events in donors will be recorded in the CRFs and reported to the IRB when necessary. The following are expected donor outcomes that will not be reported to the IRB unless they meet the criteria of an SAE:

•Common side effects of filgrastim (G-CSF) administration: bone pain, fatigue, arthralgias, headache, insomnia, fever, worsening of pre-existing skin rashes, increases of alkaline phosphatase, lactate dehydrogenase and/or uric acid levels, elevated blood leukocyte count, or thrombocytopenia.
•Hypotension during apheresis
•Hospital admission to safeguard a catheter

The following are expected donor outcomes that will be reported in summary form at the time of continuing review, but will not be reported to IRB at each occurrence unless meeting the criteria of an SAE:

•Ischemic chest pain during filgrastim (G-CSF) administration
•Splenic enlargement
•Cutaneous vasculitis
•Bone pain, muscle aches or headaches requiring narcotic analgesics

6.3 Adverse Events in HCT Recipients

The following are expected transplant recipient outcomes and will be collected from Day 0 to Day 42 and documented in the subject's medical record but not reported to IRB unless they meet the criteria for an SAE:

- Renal insufficiency
- Hepatic insufficiency
- Transient cardiac arrhythmias
- Transient cardiac insufficiency
- Pulmonary insufficiency
- Neutropenia and its complications
- Thrombocytopenia and its complications
- Anemia and its complications
- Transfusion reactions
- Treatable infections from bacteria, viruses, protozoa and fungi
- Late effects of transplant regimens including: chronic fatigue, cataracts, infertility, growth impairment, hypothyroidism, bone complications, and dental caries
- Headache, insomnia, psychosis, mood changes, disorientation, metabolic imbalance
- Nausea, vomiting, diarrhea, mucositis, weight loss, dry mouth, hiccoughs, constipation
- Well-characterized drug reactions - allergic manifestations, "red man" syndrome, steroid effects
- Well-characterized drug side effects from drugs used routinely in transplant recipients (e.g.; preparative regimen chemotherapy, immunosuppressive drugs, antimicrobials)

- Common side effects of antiemetics, analgesics, anti-inflammatory agent and known complications of steroid therapy
- Complications from intravenous catheters, thrombotic occlusion, infection, local reactions, cardiac arrhythmia
- Expected adverse events related to investigational reagents and transplant drugs are listed in section 13.3.

The following are expected transplant outcomes that will be reported in summary form at the time of continuing review but not be reported to IRB at each occurrence unless they meet the SAE criteria:

- Acute graft-versus-host disease
- Chronic graft-versus-host disease
- Graft failure / graft rejection
- Veno-occlusive disease
- Hemorrhagic cystitis
- Cytomegalovirus reactivation or disease
- EBV reactivation or disease
- Autoimmune phenomenon
- Fungal infections
- Disease relapse or progression

6.4 Expected Toxicities to Triplex

Expected toxicities associated with Triplex in healthy volunteers and HCT recipients include cutaneous reaction (grade 3), Myalgia (grade 1-2), Malaise (grade 1-2) and Headache (grade 1-2). In addition, bruising at the site of injection and transient hypotension are possible post vaccinationAll SAE data will be collected throughout the duration of the study, for up to one year.

7.0 AGENT INFORMATION

7.1 CMV-MVA Triplex

The Triplex vaccine is a multiple-antigen recombinant MVA with genes encoding 3 major CMV proteins: UL83 (pp65), UL123 (IE1), and UL122 (IE2). The Triplex vaccine was manufactured at COH CBG, in a California Food and Drug Branch (CFDB) licensed manufacturing facility which operates under the principles of GMP for the manufacture of phase I/II biologics. 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 GMP Triplex vaccine was performed at the Southern Research Institute (Birmingham, AL; Study # 13928.01.01). The IND is held by COH (BB-IND #15792. Triplex vaccine is supplied frozen at approximately 9.1 $x10^8$ pfu/mL/vial in the formulation buffer of PBS, 7.5% lactose.

7.1.1 GMP Triplex Production

CEF cells are seeded at a density of 4.9 x 10^4 cells/cm^2in T225 flasks containing complete VP-SFM Media (Life Sciences)and incubated for ~96 hours at 37°C, 95% humidity and 5% CO_2. The total number of viable cells are determined from one flask using trypan blue exclusion. The media is replaced for the control

flasks and the remaining flasks infected at an MOI of 0.02 using the Master Viral Seed Stock (MVSS), Batch# 0825-181-0001. Each flask, containing approximately 9.2 x 10^6 cells, is infected with 1.8 x 10^5pfu of MVSS, with Cytopathic effect observed ~48 hours post-infection. ~4L per sub-batch of the harvested crude cell suspension is collected and ~280 mL of sample from each sub-batch collected for QC testing. The remaining volume is centrifuged for 15' at 1500 rpm (491xg) using a Sorvall RT-7 centrifuge. Cell pellets are collected and frozen in a -80°C freezer for up to 96 hours prior to purification. Purification of Triplex from each sub-batch pellet is performed on separate days. Virus-infected pellets are thawed, resuspended in 84 mL of 10 mMTris-HCL, pH 9.0 and homogenized on ice, using 100 a 40 mL Dounce Tissue grinder. The homogenized cell suspension are sonicated twice for 30" (using one second pulse cycles), being placed on ice between each round of sonication. The homogenate is then centrifuged for 10' at 1600 rpm (558 xg) using a Sorvall RT-7 centrifuge to remove cell debris. A 30'-45' Benzonase® incubation step, using 500 units of enzyme per mL of supernatant is performed at 37°C. The virus suspension is then layered in ultracentrifuge tubes containing 15 mL of 36% sucrose and spun at 32,000xg using a Beckman Optima L90K for 80 minutes at 4°C. The effluent is removed and subsequent washes of the pellet performed. The wash step includes reconstitution of the pellet in 1mM Tris-HCl, pH 9.0 and ultracentrifugation for 60' at 4°C, 32,000xg. After the second and final wash, the effluent is removed and the viral pellets reconstituted in 7.5% Lactose/PBS. Each sub-batch will be tested for sterility. Clinical lots are prepared by thawing the bulk product containers at room temperature and pooling four purified sub-batches. The prepared pooled bulk is diluted to achieve a final concentration of 5.0-6.0 x 10^8pfu/mL in 7.5% lactose/PBS.

Storage and disposal: Triplex is to be stored in a monitored freezer between -70 to -90 °C with short term excursions up to seven days to as high as -60 °C. Stability analyses of Triplex are performed every 12 months. 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 COH or disposed of according to approved institutional standard practice. Triplex is considered a BSL 1+ reagent. No unused agent will be disposed of without prior approval by Dr. Don Diamond (ddiamond@coh.org).

7.1.1 Preparation of Triplex for Injection

Triplex will be prepared for injection at the COH Investigational Drug Service

Reagents and equipment required:

- Triplex vaccine vial (approximately 9.1x10^8 pfu/mL)
- PBS containing 7.5% lactose, fill volume 1mL
- Vortex
- Test tube cooling block
- NIST thermometer
- Clinical centrifuge
- Sterile syringes (1mL and 3mL) and needles
- Small sealable amber plastic bag
- Alcohol swabs
- Container with Ice packs

Note: the Triplex vaccine must be administered within 10 hours after the Triplex vaccine has thawed.

Preparation Procedure:

- 30 minutes before the scheduled vaccination, obtain Triplex vaccine vial from -70°C freezer.
- Put cooling block in hood and set to 4-8°C. Once the LCD (Liquid Crystal Display) on the cooling block reads 4-8°C, measure the temperature of the cooling block with the NIST thermometer (take the NIST thermometer out of the freon and lay it on the block). Adjust LCD until NIST thermometer reads 4-8°C. Only

the National Institute of Standards and Technology (NIST) or digital 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.
- Preparation of vaccine must be done at approximately 4-8 ˚C. Thoroughly wipe the exterior of the vial 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 0.9-1mL of vaccine into an appropriately sized sterile syringe.
- Record the time that the Triplex vaccine is placed in a container with ice packs and is ready for transport.
- After labeling appropriately, place syringe in sealable, plastic bag and place in container with ice packs.
- The time by which the vaccine needs to be administered – start time +10 hours – must be clearly noted in order to inform the person administering the vaccine (e.g. “Administer before xx:xx am/pm”).

8.0 CORRELATIVE STUDIES

8.1 Immunogenicity testing

Research blood draws will be collected at regular points (see study calendar) to measure CMV-specific CTL. A volume of approximately 30 mL of blood in heparin (green-top) tubes, gently inverted several times to mix anti-coagulant, and kept at room temperature until transport to Dr. Don Diamond's laboratory at COH. **If the Day 5** (day -1/0 donor sample) **donor research blood draw is not collected, residual PBSC will be used as an alternative to quantify the frequency of CMV-specific T cells post vaccination.**

PBMC will be separated from heparinized blood by standard density gradient centrifugation methods, washed, re-suspended in fetal calf serum (FCS) with 10% DMSO, aliquoted at approximately 5 million cells per aliquot and cryopreserved in centrally monitored liquid nitrogen tanks. Samples will be labeled with the study timepoint (e.g. Day 42), date of collection, study protocol number and participant study number. The correlative immunogenicity studies conducted in Dr. Diamond’s laboratory 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.

8.2 MVA vector persistence

Previous studies have shown that only minimal levels of MVA DNA were detectable in the circulation of healthy volunteers after vaccination with 10^8 pfu of Triplex [135]. HCT donors are considered equivalent health wise to healthy volunteers and hence will not be monitored for MVA persistence post vaccination.

The chance of MVA DNA being transferred from vaccinated donors to HCT-R via the PBSCH is small. However, the persistence of MVA DNA in the blood of HCT-R will be monitored during the 12 month observation period after transplant.

A tube of 3 mL of blood in citrate (yellow top) will be collected for this evaluation at times specified in the study calendar. The method used to detect residual, circulating MVA DNA was validated by the Quality Assurance Department of COH and used to monitor vector persistence in healthy volunteers (COH

IRB#08173). This real-time PCR approach employs separate sets of primers for the MVA backbone and the CMV insert genes utilizing TaqMan™ reagents and is sensitive to<20 copies of MVA DNA per sample. A plasmid DNA standard is employed to quantify the copy number of MVA and insert genes detected in blood specimens. Analysis will be performed in a single batch at end of the study after all Triplex vaccinations have been completed.

9.0 STUDY CALENDAR

HCT Donor:	Pre	Days						
Study day	study	-60 to -10	-5 to-1	-1/0[c]	d40	d90	d180	d365
Informed Consent/ Registration	X							
G-CSF			X -------- X					
CMV-MVA Triplex Vaccine administration		X[a]						
AE assessment*		X	X	X				
Apheresis				X				
Research blood sample: *30 mL Heparin*		X[f]		X[a]	x[b,e,f]	X[b,e,f]	X[b,e,f]	X[b,e,f]
Pregnancy Test (if applicable)[H]		X						

[a]research blood draw to be collected prior to vaccination/apheresis [b]donor blood draws day 40-365 are optional. [H]Urine pregnancy test to be performed for females of child bearing potential prior to vaccine. *The donor will be called within 7 days of vaccination to record any adverse reactions.

[C] -1/0 donor sample blood draw corresponds to day 5 donor research blood draw

d (+/-) 15 day window

e draws are based on 40, 90, 180, 365 days post the actual vaccine injection day

f draws include CBC with differential

Patient (HCT recipient):	Pre-HCT	HCT at Day 0*		Post HCT assessments (Days)									
Study day			14	[c]28	[c]42	c56	c70	[c]84	[c]100	[d]140	[d]180	[d]270	[d]365
Myeloablation	X												
Informed consent/enrollment	X												
Eligibility/screening tests	X												
Pregnancy test (if applicable)	X												
AE (CTCAE) assessment			X	X	X								
CMV qPCR				X	X	X	X	X	X	X[e]----------X[e]			
KPS performance status	X			X		X							
Concurrent medications[f]	X			X		X		X---------- X					
Physical exam and vital signs[h]	X			X		X		X---------- X					
Engraftment status				X	X	X	X	X ---------- X					
Disease relapse assessment				X		X		X ---------- X					
GVHD assessment/grading				X		X		X ---------- X					
CMV disease assessment				X	X	X	X	X ---------- X					
Chemistry/metabolic panel[h]	X			X		X		X ---------- X					
CBC with differential				X	X	X	X	X	X	X	X	X	X

CMV-MVA Triplex Vaccine administration (Arm 2 only)				X		X							
Research blood sample[g]: *30 mL heparin tube (immunogencity)* *3 mL citrate (MVA DNA)*				X X	X	X X	X	X	X X	X X	X X	X X	X X

[c] Post-HCT assessments/procedures permitted +/- 5 days. [d] Post-100 Day visits permitted +/- 15 days.[e] Post Day 100 CMV qPCR only if clinically indicated. [f] concurrent medications data to be collected in CRFs: duration of anti viral medications, immunosuppressive agents, steroid doses/duration and prohibited medications. [g]Research blood samples to be collected at the same time as SOC blood draws when possible. X----X denotes frequency as per SOC/institutional practice or PI/treating physician's discretion. [h] include those listed in Section 5.2.5

10.0 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

The primary objective is to establish the feasibility and safety of priming CMV immunity in donors by Triplex vaccination prior to PBSC harvest. The hypothesis is that it is feasible and safe to HCT donors with the Triplex vaccine and that vaccination increases the frequency of CMV-specific T cells in both donors and recipients.

Primary endpoints:

Feasibility: a record will be made of the number of suitable donor/recipient pairs were approached, and the number that were successfully enrolled/vaccinated.

Safety endpoints: AEs ≥ grade 2, probably or definitely related to vaccination will be noted in donors. Non-relapse mortality (NRM) at 100 days post HCT, delayed engraftment, severe aGVHD and grade 3-4 AEs (CTCAE v.4.0) probably or definitely related to the intervention will be assessed in recipients.

Therapy induced quantitative/kinetic changes in CMV-specific cellular immunity: T cells specific for pp65 and/or IE antigens will be measured as a possible correlate to protective function. The frequency of T cells specific for pp65 and/or IE antigens will be measured using CD137 expression assays. These CMV-specific T cells will be further characterized by CD107-associated degranulation, polyfunctional cytokines and cell-surface memory markers.

Secondary endpoints:

CMV protection: incidence of viremia (≥1250 IU/mL), CMV viral load and use of antivirals (recipients who reactivate CMV *and* are given antiviral therapy will be considered intervention failures).

11.0 STATISTICAL CONSIDERATIONS

11.1 Number of Subjects and Accrual

For each arm, eighteen CMV-P transplant recipients will be enrolled, along with their donors at COH and DFCI. We anticipate about 100 recipient/donor pairs to be eligible annually at COH. Accrual should be completed in 4 years from the start date of the trial, and we anticipate 1 year of follow up and data analysis.

11.2 Objective

The aim of this study is to examine the feasibility, safety and immunogenicity of Triplex vaccination in HCT donors prior to apheresis for PBSCH. We will also evaluate safety and CMV-specific immunity in the recipients. Due to the sample size limitations of a pilot study, assessment of clinical efficacy will be exploratory.

Vaccination will be suspended for safety review if there is evidence of serious treatment-related AEs. Specifically:

(1) Donors will be observed on the day of vaccination, and at apheresis. Any AEs attributed to Triplex vaccination will be reported to the DSMC. In a study of Triplex vaccination in healthy volunteers, vaccination was well tolerated, and the same is expected in HCT-D who are equivalent health wise. However, HCT-D will also be mobilized with GSCF for 5 days after vaccination. The potential risk of combined toxicity from vaccination and GSCF are considered small, but must be explored.

(2) 100 days non-relapse mortality (NRM).

(3) Severe acute GVHD (aGVHD, grade 3-4) will be monitored on a per patient basis. The trial will be interrupted if 2 or more of the first 3 or 4 recipients, or if any 3 recipients experience Grade 3-4 aGVHD. This would be a significant elevation from the COH historical benchmark of 15% of allogeneic HCT recipients with matched sibling donors [1].

(4) Serious AEs (SAE, grade 3-4) probably or possibly related to the experimental therapy will be individually reviewed by the protocol monitoring team, and reported to the DSMC.

(5) Delayed engraftment will be monitored

(6) Donors will be observed on the day of vaccination and at apheresis.

11.1 Data Analysis Plan

Feasibility: the number of donors vaccinated as a percentage of the total donor-recipient pairs enrolled (i.e. a consenting recipient) will be calculated. With 18 donor/recipient pairs per arm, the standard error of the percentage at most will be 0.12 with a margin of error of 0.24

Cellular immunity: the frequency of Triplex expanded CMV specific T cells in donors will be assessed by comparing pre-vaccine and pre-apheresis blood draws. The previous study of Triplex vaccination in healthy volunteers (COH IRB#08173) showed a notable expansion of CMV specific T cells after one vaccination. HCT donors are equivalent health wise to the volunteers on this study, hence are expected to respond immunologically.

The frequency of CMV specific T cells in donors after apheresis will be assessed from day 28 onwards, until day 365 where possible. The data analysis for estimating the effect of donor vaccination on functional cellular immunity through time will be exploratory in nature, and will focus on graphical display and summary statistics. T cells specific for pp65 and/or IE antigens will be measured as a possible correlate to protective function.

Clinical Efficacy (CMV Events): Each recipient will be followed for the occurrence of CMV reactivation events, including CMV viremia, the use of CMV-directed antiviral therapy, or detection of CMV disease. Both initial and recurrent events will be recorded, with patients considered at risk for recurrent events after completion of a full planned course of anti-viral therapy. The total days on antivirals for CMV reactivation (induction, maintenance, and total) will be assessed for each individual. Evidence of clinical

efficacy will be assessed within the sample size limitations of a pilot study. The target of 18 donor/recipient pairs, gives an 80% power (0.10 significance) to detect an improvement in CMV reactivation rate from 30% to 10%. Two or fewer reactivations would indicate a positive effect of the intervention (relative to the 30% benchmark) at the 0.06 significance level. Patients receiving HCT from vaccinated donors can be compared to historical controls of patients receiving HCT from unvaccinated donors. This may provide a preliminary estimate of the value of vaccinating HCT-D, with regard to protection from reactivation.

12.0 DATA HANDLING, DATA MANAGEMENT, RECORD KEEPING

12.1 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.

12.2 Data Capture Methods and Management

Data for this trial will be collected using electronic capture at COH that is 21CRF Part 11 compliant.

Study personnel 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.

12.3 Case Report Forms

Study personnel 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.

13.0 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 institutional procedures to the local IRB and DSMC.

13.1.1 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 Principal Investigator must be notified as soon as practicable (within 24 hours) via email, providing the following:

- Description of the event
- Impact on participant safety or the safety to others
- Impact on the study design

13.1.2 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.

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)

14.0 STUDY OVERSIGHT, QUALITY ASSURANCE AND DATA & SAFETY MONITORING

14.1 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 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 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.

14.2 Monitoring

The Investigator/Institution will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

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

15.0 ETHICAL AND REGULATORY CONSIDERATIONS

15.1 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.

15.2 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

15.3 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 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.

15.4 Informed Consent

After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative before study participation. HCT donors undergoing Triplex vaccination will also be required to sign an ICF document. The method of obtaining and documenting the informed consent and the contents of the consent must comply with the ICH-GCP and all applicable regulatory requirements.

Before implementing any study procedure, informed consent shall be documented by the use of a written consent form approved by the IRB and signed and dated by the patient or the patient's legally authorized representative at the time of consent. A copy of the signed informed consent will be given to the patient/donor or their 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.

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

15.5.1 Inclusion of Women and Minorities

The study is open anyone regardless of gender or ethnicity and efforts will be made to extend the accrual to a representative population. If differences in outcome that correlate to gender 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 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 vaccination of the mother, breastfeeding should be discontinued if the mother is enrolled on this study.

15.5.2 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, cell/vaccine dosage and the blood

volume established for immune-monitoring in adults is not applicable to children. Finally, the risk of CMV complications is inversely related to age, and the inclusion of younger children could bias the endpoint observations.

15.5.3 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 HIV negative individuals.

15.6 Participant Confidentiality

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

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

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

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

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

15.7 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 investigators will follow their institutional conflict of interest policy.

15.8 Financial Obligations, Compensation, and Reimbursement of Participants

The investigational agents will be provided free of charge. 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 HCT recipient and/or the insurance carrier. The HCT-R will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The HCT-R 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 study participant (recipient or donor) resulting from research procedures, appropriate medical treatment will be available. City of Hope will not provide financial compensation in the event of physical injury to a research participant.

HCT recipients will not receive reimbursement or payment for taking part in this study.

HCT donors will be paid $50 for each blood draw donated at time points after apheresis. The maximum number of specimens eligible for payment are 4, collected around day 40, 90, 180, and 365 (see study calendar).

15.9 Publication/Data Sharing

No part of the results of the study carried out under this protocol will be published or passed on to any third party without the written approval of the PI. Any investigator involved with this study is obligated to provide the PI with complete test results and all data derived from the study.

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

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

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

The 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: KPS scale

KPS Status	KPS Grade
Normal, no complaints	100
Able to carry on normal activities. Minor signs or symptoms of disease	90
Normal activity with effort	80
Care for self. Unable to carry on normal activity or to do active work	70
Requires occasional assistance, but able to care for most of his needs	60
Requires considerable assistance and frequent medical care	50
Disabled. Requires special care and assistance	40
Severly disabled. Hospitalisation indicated though death nonimminent	30
Very sick. Hospitalisation necessary. Active supportive treatment necessary	20
Moribund	10
Dead	0