CITY OF HOPE MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010

Department of Hematology and Hematopoietic Cell Transplantation

TITLE: A PHASE II RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER TRIAL TO EVALUATE PROTECTIVE FUNCTION OF AN OPTIMIZED DOSE OF CMVPepVax IN RECIPIENTS OF AN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 13494 VERSION: 28

DATE(S) OF AMENDMENTS/REVISIONS:

COH Initial Approval	Protocol Dated 03/30/15	Version 00
COH Amendment 01	Title Page Dated 04/22/15	Version 01
COH Amendment 02	Protocol Dated 04/28/15	Version 02
COH Amendment 03	Protocol Dated 05/05/15	Version 03
COH Amendment 04	Title Page Dated 05/26/15	Version 04
COH Amendment 05	Title Page Dated 06/09/15	Version 05
COH Amendment 06	Protocol/TP Dated 08/10/15	Version 06
COH Amendment 07	Protocol/TP Dated 09/01/15	Version 07
COH Amendment 08	Protocol/TP Dated 11/09/15	Version 08
COH Amendment 09	Protocol/TP Dated 12/08/15	Version 09
COH Amendment 10	Protocol/TP Dated 1/19/16	Version 10
COH Amendment 11	TP only Dated 02/02/16	Version 11
COH Amendment 12	TP/Protocol Dated 03/08/16	Version 12
COH Amendment 13	TP/Protocol Dated 04/12/16	Version 13
COH Amendment 14	TP/Protocol Dated 05/12/16	Version 14
COH Amendment 15	TP/Protocol Dated 06/09/16	Version 15
COH Amendment 16	TP/Protocol Dated 07/12/16	Version 16
COH Amendment 17	TP/Protocol Dated 10/24/16	Version 17
COH Amendment 18	TP/Protocol Dated 11/01/16	Version 18
COH Amendment 19	TP/Protocol Dated 12/21/16	Version 19
COH Amendment 20	Title Page Dated 04/25/17	Version 20
COH Amendment 21	TP/Protocol Dated 04/03/17	Version 21
COH Amendment 22	TP/Protocol Dated 07/14/17	Version 22
COH Amendment 23	TP/Protocol Dated 10/03/17	Version 23
COH Amendment 24	TP/Protocol Dated 11/01/17	Version 24
COH Amendment 25	TP/Protocol Dated 04/08/2019	Version 25
COH Amendment 26	TP only Dated 05/15/2019	Version 26
COH Amendment 27	TP only Dated 07/02/2019	Version 27
COH Amendment 28	TP/Protocol Dated 02/19/2020	Version 28

DISEASE SITE: Hematopoietic System
SPONSOR/IND NUMBER: City of Hope / IND# BB-13124

TYPE (*e.g., Pilot, Phase I, etc.*)**:** Phase II, Therapeutic
PRINCIPAL INVESTIGATOR: Ryotaro Nakamura, M.D. *(Study PI)*, Armin Rashidi, M.D. (UMN *Site PI)*
COLLABORATING INVESTIGATORS: Don J. Diamond Ph.D., Corinna La Rosa Ph.D., Joycelynne Palmer, Ph.D.

PARTICIPATING CLINICIANS: Haris Ali M.D**.,** Ibrahim Aldoss, M.D., Shukaib Arslan, M.D., Andrew Artz, M.D., Elizabeth Budde, M.D., Ji-Lian Cai, M.D., Thai Cao M.D., Len Farol M.D., Stephen Forman, M.D., Alex Herrera M.D., Myo Htut M.D., Chatchada Karanes M.D., Samer Khaled M.D., Paul Koller, M.D., Amrita Krishnan M.D., Guido Marcucci, M.D., Matthew Mei, M.D., Al Malki M.D., Joshua Mansour, M.D., Auayporn Nademanee M.D., Liana Nikolaenko, M.D., Leslie Popplewell M.D., Vinod Pullarkat M.d., Firoozeh Sahebi M.D., Amandeep Salhotra M.D., Karamjeet Sandhu, M.D., Tanya Siddiqi, M.D., Eileen Smith M.D., David Snyder M.D., Ricardo Spielberger M.D., Anthony Stein M.D., Michael Rosenzweig M.D, Ahmed Aribi M.D, Nitya Nathwani M.D., John Zaia, M.D. Jasmine Zain, M.D.

STUDY STATISTICIAN: Dongyun Yang, Ph.D.

PARTICIPATING SITES: City of Hope, Duarte, CA (Lead Institution), University of Minnesota Masonic Cancer Center, MN, Fred Hutchinson Cancer Research Center, WA, Ohio State University, OH, Emory University, GA.

CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010

DEPARTMENT OF HEMATOLOGIC ONCOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: A PHASE II RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER TRIAL TO EVALUATE PROTECTIVE FUNCTION OF AN OPTIMIZED DOSE OF CMVPepVax IN RECIPIENTS OF AN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT

CITY OF HOPE PROTOCOL NUMBER: 13494 **VERSION:** 28

SPONSOR/IND NUMBER: City of Hope / IND# BB-13124

PRINCIPAL INVESTIGATORS

Ryotaro Nakamura M.D. (*Study PI; Lead Institution PI)*
Department of Hematology and HCT
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA
Phone: 626-256-4673
Fax: 626-301-8116
e-mail: RNakamura@coh.org

Armin Rashidi, M.D (Site PI)
Division of Hematology, Oncology, and Transplantation
University of Minnesota, Department of Medicine
14-152 Phillips Wangensteen Building
516 Delaware Street SE
Minneapolis, MN 55455
Phone: 612-624-0123
Fax: 612-625-6919
Email: arashidi@umn.edu

Joshua Hill, M.D. (Site PI)
Vaccine and Infectious Disease Division
Fred Hutchinson Cancer Research Center
1100 Fairview Ave. N, Seattle, WA
Phone: 206-667-6504
Fax: 206-667-4411
Email: jahill@fredhutch.org

Hannah Choe, M.D. (Site PI)
Ohio State University,
B316 Starling Loving Hall
320 W 10th Avenue, Columbus, Ohio 43210
Phone: 614-293-5655
Email: Hannah.Choe@osumc.edu

Ned Waller, M.D., PhD (Site PI)
Department of Hematology and Medical Oncology,

Winship Cancer Institute, Emory University,
Bldg C, 1365 Clifton Road NE, Atlanta, GA 30322
Phone: (404) 778-1900
Fax: (404) 778-3110
Email: ewaller@emory.edu

COLLABORATING INVESTIGATORS

Don J. Diamond, Ph.D.
Department of Hematology and HCT
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA
Phone: 626-256-4673
Fax: 626-301-8981
e-mail: DDiamond@coh.org

Corinna La Rosa, Ph.D.
Department of Hematology and HCT
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA
Phone: 626-256-4673
Fax: 626-301-8981
e-mail: Clarosa@coh.org

Jeffrey S. Miller, M.D.
University of Minnesota Medical Center
Medicine/Blood and Marrow Transplantation
425 E. River Rd, CCRB 654
Minneapolis, MN 55455
Phone: 612-626-4024
Fax: 612-626-3941
e-mail: mille011@umn.edu

STUDY STATISTICIAN:

Jeff Longmate, Ph.D.
Department of Information Sciences
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA
Phone: 626-256-4673
Fax: 626-471-7106
e-mail: JLongmate@coh.org

PARTICIPATING SITES:

City of Hope, Duarte, CA (Lead Institution)

Participating sites:

University of Minnesota Masonic Cancer Center, MN

Fred Hutchinson Cancer Research Center, WA,

Ohio State University, OH.

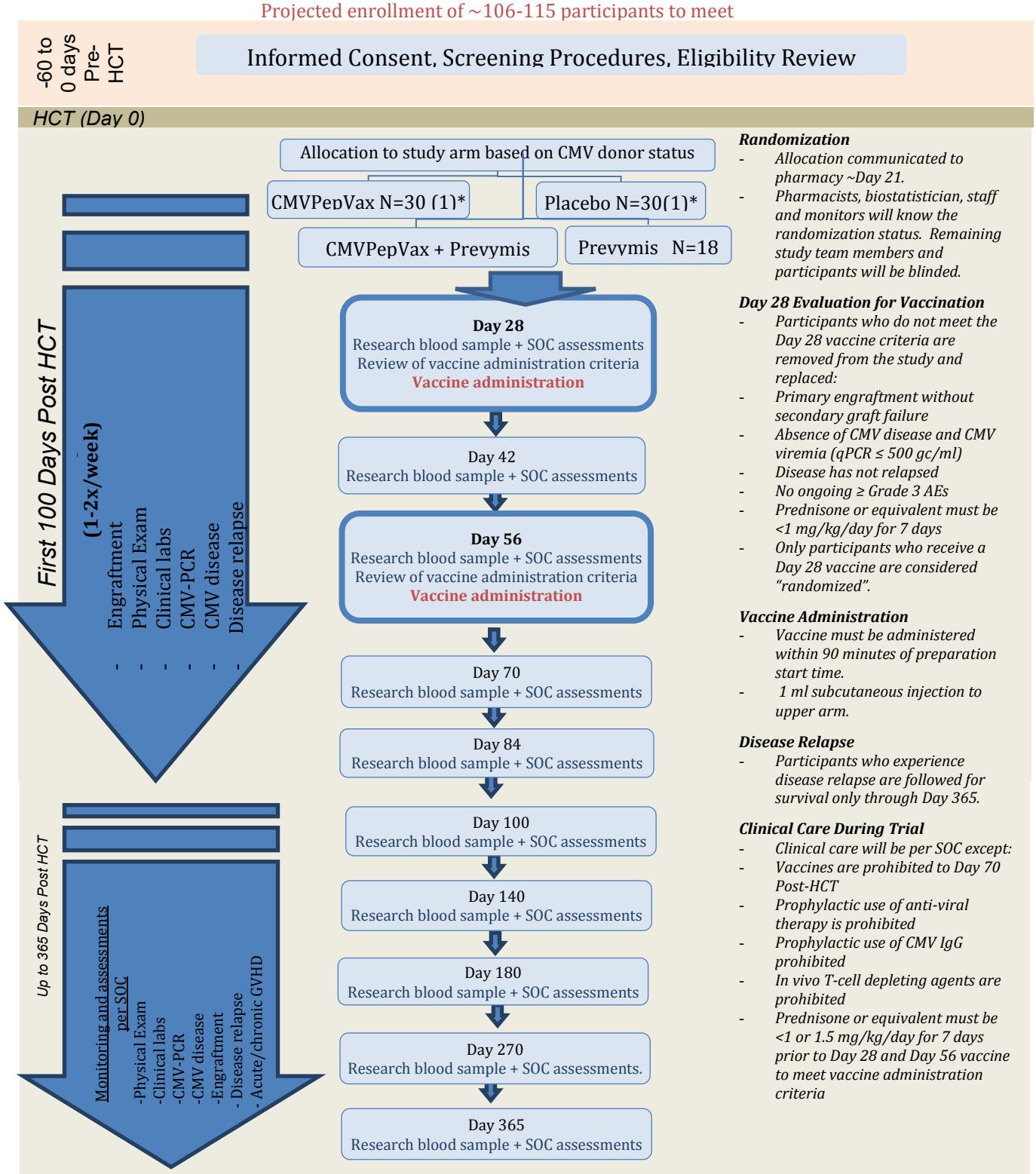
Emory University, Atlanta, GA.

AGENT NSC#	NSC# 721434
COORDINATING CENTER	Data Coordinating Center City of Hope National Medical Center 1500 E. Duarte Road Duarte, CA Phone: 626-256-4673 x 63968 e-mail: DCC@coh.org

Experimental Design Schema

CMV$^+$ adults undergoing 8/8 high resolution HLA donor allele matched hematopoeitic stem cell transplant (HCT) for treatment of a hematologic malignancy

Projected enrollment of ~106-115 participants to meet



* The first cohort consisting of 61 patients is still blinded, so it is not known which arm has 30, and which 31 patients

Summary of the amendment

The protocol is being revised with inclusion of an additional cohort that would allow testing a combination of the CMVPepVax vaccine and a newly approved anti-viral agent Prevymis (letermovir) in order to address possible synergy between CMV suppression by Prevymis and boosting anti-CMV immunity by CMVPepVax vaccine. For this purpose the accrual of the cohort including 61 patients not administered Prevymis is closed, and accrual of a new cohort of 36 patients is proposed where all patients would be administered Prevymis and co-administered CMVPepVax or placebo (blinded and randomized 1:1).

This new design will allow addressing the unmet need of stimulating CMV-specific immunity and lowering reactivation risk for patients after cessation of Prevymis dosing.

Protocol Synopsis

Protocol Title:
A Phase II randomized, placebo-controlled, multicenter trial to evaluate protective function of an optimized dose of CMVPepVax in recipients of an allogeneic HSCT.
Brief Protocol Title for the Lay Public (if applicable):
CMVPepVax, a new vaccine to prevent cytomegalovirus infection after allogeneic stem cell transplantation
Sponsor, IND
COH under IND# BB-13124
Study Phase:
Phase II, Randomized, Blinded, Interventional
Participating Sites:
City of Hope National Medical Center, University of Minnesota, Fred Hutchinson Cancer Research Center, Ohio State University, Emory University
Rationale for this Study:
Cytomegalovirus (CMV) reactivation with ensuing viremia is one of the main infectious complications in the first 100 days following allogeneic hematopoietic cell transplant (HCT) [1-6]. While anti-viral agents that limit viral replication may keep CMV viremia initially in check, they may also result in significant toxicity and fail to protect against late-onset CMV disease, including reactivation and failure to reconstitute CMV-specific immunity. CMVPepVax (IND BB-13124) is a peptide vaccine composed of an antigenic HLA A*0201 restricted $pp65_{495-503}$ CMV CD8 cytotoxic T cell (CTL) epitope, covalently linked to a universal tetanus T helper (TH) epitope [7] and co-administered with Pfizer PF-03512676 adjuvant [8]. HLA-restricted peptide epitopes are a promising option to develop a safe, non-infectious subunit CMV vaccine, avoiding CMV immune-evasion mechanisms [9]. Moreover, it has been reported that T cells specific for CMV-pp65, a principal target for CTLs can protect HCT recipients from CMV complications [9, 10]. The PF-03512676 adjuvant is a single strand DNA-based sequence containing bacterial CpG DNA motifs, which activates Toll-like Receptor 9 (TLR9) leading to potent immunostimulatory effects, such as B cell and NK cell

activation and proinflammatory cytokine production.

A Phase Ib trial conducted in HLA A*0201 healthy adults (COH IRB 03121) indicates that CMVPepVax is safe and induces robust expansion of CMV-pp65 CD8 T cells in the healthy adult population [8]. A second Phase 1b pilot trial (COH IRB 12022) was conducted in CMV seropositive HLA A*0201 adult recipients of matched related donor (MRD) or unrelated donor (MUD) HCT. A total of 36 patients were randomized to Vaccine Arm (VA) or Observation Arm (OA); those who were assigned to VA received CMVPepVax on days 28 and 56 post-HCT and those in OA were followed for clinical and immunologic data monitoring (open label).

Preliminary results from this target population indicate excellent tolerability, safety and immunogenicity of CMVPepVax compared to the observation arm. CMV viremia (DNAemia) was observed in 6 of 18 patients in OA compared with 1 of 18 patients in VA. Furthermore preliminary data from this study suggest that CMVPepVax may improve transplant related outcomes such as chronic GVHD (cGVHD) and disease relapse. Now that safety and preliminary immunogenicity have been established in the target population with the day 28 and 56 post-HCT vaccination schedule, a Phase II study is necessary to assess the efficacy of CMVPepVax administered with this schedule and in the target population to assess efficacy in protecting against CMV reactivation and disease.

Rationale for the revised aims and design based on the interim analysis:

The interim analysis (after 48 patients reached day 100 follow up) for the primary endpoint (Section 12.4 Data Analysis Plan) unexpectedly showed that more CMV events observed on the vaccine arm (5 events) than on the placebo arm (3 events), thus meeting the futility stopping rule. Additional data on these 48 patients (24 in CMVPepVax arm, 24 in placebo arm) are detailed in Table 2.5. While the clinical efficacy was not seen for the primary endpoint, the immunologic recovery was favorable for the CMVPepVax arm, and no safety issues have been found. Taking the abovementioned finding into account the cohort of patients that were enrolled initially (61 patient) is now closed for accrual. Anti-viral agent Prevymis was approved for CMV prophylaxis after the study was initiated, and while CMVPepVax is not currently thought to be a suitable alternative for Prevymis administration, it can possibly address the significant risk of CMV reactivation that occurs after the completion of Prevymis administration [11]. This unmet need motivated the amendment of the protocol for the study to include an additional cohort, which could verify a possible synergy in treatment of the patients with a combination of Prevymis and CMVPepVax. Complementing antivirals with a vaccine that harnesses the abundant native immune response to CMV may improve outcomes for HCT recipients [12]. In addition, this approach may allow achieving protective immunity ahead of the early reactivation that may occur during the prophylaxis period and was observed in the absence of Prevymis prophylaxis. Because 7 of the 8 primary outcomes occurred prior to the second planned injection, the rationale for using CMVPepVax in the context of Prevymis prophylaxis is thought to be directly relevant to the nature of the events that led to early stopping.

Objectives:

Primary Objective:
For the entire cohort (n=97):
1) To determine if CMVPepVax increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated HLA A*0201, CMV seropositive HCT-recipients,

For the Prevymis combination cohort (n=36)

1) To provide a preliminary evaluation of the incidence of CMV reactivation between day 28 and day 180 in patients who receive standard Prevymis prophylaxis (from day 14 through day 100), comparable to the evaluation of an expansion cohort in a pilot study, or the futility stage of a phase II trial.

2) To determine if CMVPepVax increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated HCT patients who receive standard Prevymis prophylaxis.

Secondary Objectives:

1. To determine, within the constraints of a pilot cohort, if CMVPepVax reduces the frequency of CMV events alone or in combination with Prevymis defined as reactivation or CMV disease in HLA A*0201 allogeneic HCT-R+. A CMV event encompasses any detection of CMV by either qPCR (termed "reactivation": DNAemia ≥500 gc/ml or by tissue histology (end-organ disease).
2. To evaluate the safety and tolerability of CMVPepVax by assessing the following: non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and grade 3-4 AEs (CTCAE 4.0) probably or definitely related to the vaccination within 2 weeks from each vaccination.
3. To characterize CMV reactivation and CMV disease in recipients of CMVPepVax compared to placebo by assessing time to viremia (defined as number of days from transplantation to the date of ≥500 CMV gc/mL), duration of viremia, recurrence of viremia, incidence of late CMV viremia/disease (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia, cumulative number of CMV specific antiviral treatment days. To evaluate the impact of CMVPepVax on transplant related outcomes by assessing the incidence of acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, non-CMV infections.
4. To determine whether vaccination induces adaptive NK cell population changes, and increase in the highly cytotoxic memory NKG2C+ NK cells,
5. To determine the impact of CMVPepvax on CMV immune reconstitution in patients who undergo treatment with antiviral agent Prevymis
6. To explore GVHD biomarkers and compare between the vaccine and placebo groups,
7. To characterize CMV reactivation after day 180

Study Design:

Overall design:

This is a multi-center, randomized, blinded, placebo-controlled, parallel-group Phase II trial to evaluate: a) increase in CMV cellular immunity, in HLA A*0201 allogeneic HCT-R^+ vaccinated with CMVPepVax relative to those injected with placebo, b) reduction in the frequency of CMV reactivation and disease (CMV events). This trial is designed to 1:1 randomly allocate 97 HLA A*0201 allogeneic

HCT-R^+ at risk for CMV complications to CMVPepVax arm or placebo arm [13].

The revised protocol after the interim analysis will enroll 36 patients (18 in the CMVPepVax arm, 18 in the placebo arm) to provide an initial evaluation of vaccination in combination with Prevymis to help plan future studies if the futility endpoint is not met and T-cell results are favorable. Final immunologic analysis will include both the new (n=36) cohort as a subgroup and combined cohorts (n=97). Treatment categories for the 18 vs 18 cohort include either Prevymis + CMVPepVax, or Prevymis + Placebo. No treatment category in this cohort includes CMVPepVax only, placebo only, or CMVPepVax + placebo.

Enrollment and randomization:

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

After the interim analysis of 61 enrolled patients resulted in reaching futility in regard to the primary endpoint, the study has been amended to provide a preliminary evaluation of clinical benefit, and immunological outcomes, comparing two study arms that were added to evaluate CMVPepVax efficacy in combination with Prevymis compared to Prevymis alone. For this reason, we have closed accrual of the initial cohort (61 patients) who did not receive Prevymis and propose to enroll a new cohort of 36 patients to be assigned to either CMVPepVax, or placebo groups, and all of whom will concurrently receive Prevymis. Every patient in the new cohort of 36 patients (18 vs 18) will receive Prevymis as standard of care. The combined enrollment will be conducted up to a total of 97 patients.

Administration of CMVPepVax/Placebo: A 1 ml volume of vaccine (CMVPepVax or placebo) is administered subcutaneously (SC) on days 28 and 56 post-HCT.

Administration of Prevymis: Patients in both Prevymis arms will receive Prevymis at a dose of 480 mg per day through week 14, up to 100 days post-HCT.

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

Stopping rules and planned interim analysis: Rigorous stopping rules will be implemented, and will include three major safety endpoints; non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and serious AEs (SAE, grade 3-4) related to the vaccination within 2 weeks from each vaccination. There will be an interim futility analysis when the first 48 patients are evaluable for the primary endpoint (frequency of CMV reactivation before day 100). If the observed CMV event-rate in the CMVPepVax arm is higher than on the placebo arm, the study will be suspended for NCI and DSMC review [14]. The loss of power of invoking a stopping rule at 50% of accrual is less than 0.02 [14], under the event rates assumed for planning. In light of the lower

observed rates, the false alarm rate should be revised upward.

Monitoring of the Prevymis cohort will be done by applying the original monitoring rules for NRM, severe aGVHD, and SAE in two ways, firstly treating subjects on Prevymis and CMVPepVax as a continuation of the CMVPepVax arm, and secondly, treating subjects on Prevymis and CMVPepVax as a novel therapy and applying the monitoring rules for the first 12 subjects. There will be no further interim analysis for futility, as the entire Prevymis cohort is motivated, in part, as an opportunity to subject the combination of CMVPepVax with Prevymis to a futility evaluation.

Endpoints:

Primary endpoints:

For the entire cohort (n=97): Immunological endpoints - levels of $CD8^+$ T cells binding to A2-CMV-dextramers®, combined with T and NK cell phenotype and function.

For the combination cohort (n=36): CMV reactivation [≥1,250 IU/mL] or CMV disease between day 56 and day 180 post-HCT in patients who receive standard Prevymis prophylaxis (from day 14 through day 100).

Secondary endpoints:

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

CMV reactivation [≥1,250 IU/mL] or CMV disease prior to day 100 post-HCT. Timing and recurrence of events are included in the primary analysis.

CMV-related endpoints: Duration of viremia, incidence of late CMV viremia (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia), cumulative number of CMV specific antiviral treatment days.

Clinical endpoints: Time to engraftment, incidence of aGVHD, chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, non-CMV infections.

Immunologic endpoints for the combination cohort (n=36): Immune reconstitution measured as levels, function and kinetics of CMV-specific T cells through day 180 post-HCT in patients who receive standard Prevymis prophylaxis (day 14 through day 100).

Sample Size:

The target of this Phase II multicenter study was originally to randomize 97 HLA A*0201 allogeneic HCT-R^+ to either the CMVPepVax vaccine arm (N=48), or to the placebo arm (N=48). With the expected dropout rate of 10-15% prior to randomization, the total accrual is expected to be 106-115.

Following the results of the interim analysis the original cohort (n=61) that included patients without Prevymis administration is closed for accrual.

New cohort (Prevymis cohort) is opening for accrual in which 36 patients will be randomized 1:1 (18/18) for CMVPepVax or Placebo. The number of patients for this cohort (36 patients) is based on budgetary constraints, the futility rules for the new cohort are independent and are based on the original futility rules. Based on a Phase III trial of Prevymis, we expect 17 percent of placebo subjects to reactivate after second vaccination on week 8. This is based on the expectation that 33 percent will be high-risk subjects. Assuming that CMVPepVax vaccination during prophylaxis can reduce the week 8 through week 26 reactivation rate to 5 percent, the probability that there are more reactivations on the placebo arm than on the CMVPepVax arm is approximately 88 percent, which can be regarded as

the power for a non-futile finding.

If CMVPepVax offers no protection, there would be a 50 percent chance of a futility finding, and T-cell data would likely indicate many subjects with poor CMVPepVax antigen recognition. If CMVPepVax has strong protection (5% reactivation), a finding of futility is unlikely (12 percent) and an evident biological effect would be expected in most patients.

Estimated Duration of the Study

Over the past five years, COH and UMN combined have performed on-average ~ 300 adult allogeneic HCT procedures annually. We anticipate about 70 to be eligible annually being HLA A*0201 and CMV seropositive. It is estimated that accrual will be completed in <5 years from the start date of the trial, and that trial we anticipate 1 year of follow up and data analysis.

Summary of Subject Eligibility Criteria:

Pre-HCT Inclusion Criteria

- Age 18 to 75 years.
- Planned HCT for the treatment of hematologic malignancy (some exceptions including multiple myeloma)
- Planned related or unrelated HCT, with 8/8 (A,B,C,DRB1) HLA donor allele matching and with minimal to no T-cell depletion of graft
- HLA A*0201, CMV seropositive (recipient)
- Seronegative for HIV, HCV and active HBV

Pre-HCT Exclusion Criteria

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

Post-HCT Day 28 Vaccine Administration Criteria*

- No patients with CMV reactivation between days 0-28 post HCT, defined as having a detectable CMV level according to City of Hope institutional standard
- No ≥ Grade 3 GVHD between days 0-28 post HCT
- Disease has not relapsed since HCT
- Successful primary engraftment without secondary graft failure
- No ongoing post-HCT ≥ Grade 3 non-hem AE's, with the exception of grade 3 glucose intolerance, cholesterol, triglyceride, and hyperglycemia
- No for CMV viremia: CMV qPCR < 500 gc/ml from samples collected and resulted within the past 7 days
- Negative for CMV end organ disease (biopsy proven CMV disease) post-HCT

- All prednisone doses within the past 7 days have been ≤ 1 mg/kg/day (or prednisone equivalent)
- Not received any medications that might interfere with CMVPepVax
- For Prevymis cohort patients: patients who received Prevymis for at least 7 days before day +28 post HCT

*Participants who do not meet vaccine administration criteria will be removed from the trial and replaced. This is not an eligibility criteria for the trial.

Investigational Product Dosage and Administration:

Participants will be randomized to receive either CMVPepVax vaccine or the placebo vaccine. The pharmacy will know the randomization status of participants, while the clinical study team and participants will remain blinded. Vaccine (1 ml of placebo or CMVPepVax) will be administered subcutaneously in the upper arm on Days 28 and 56 post-HCT. Time window for the administration is +/- 7 days. There are no dose modifications, although participants must meet vaccine administration criteria in order to receive the vaccine.

CMVPepVax vaccine is constituted of approx. 2.5 mg Tet-CMV + 1.08 mg PF-03512676[8] adjuvant diluted in normal saline and sodium acetate.

Placebo vaccine comprises an isotonic solution of cGMP-grade solution of sodium acetate and Neut™ (0.4% Sodium Bicarbonate Additive Solution).

Clinical Observations and Tests to be Performed:

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

Immunologic studies: Immunologic studies will include monitoring levels of $CD8^+$ T cells binding to pp65 A2-CMV-dextramers®, combined with immunophenotyping studies [15, 16]. The phenotypic ratios of CMV-specific T cells will be related to improvement in control of CMV viremia. Highly cytotoxic memory $NKG2C^+$ NK cells [17], linked to CMV reactivation and critical for CMV adaptive immune response will be characterized at UMN [18-20].

Statistical Considerations:

Planning for measures of immune function is based on results of COH protocol 12022, the open-label analog of this placebo-controlled protocol. In that trial, 10 of 18 vaccinated subjects developed CMV pp65-specific CD8+ T-cells that were strongly concentrated (> 80%) in effector memory phenotypes, and did so without CMV reactivation. This can be compared to 1 of 14 non-vaccinated subjects who did not reactivate. In the current study, the planned sample size of 48 subjects per arm would provide approximately 80% power for a one-sided 0.05-level test assuming a 50% rate of this event from a 25% rate on the placebo arm. Beyond this measure of vaccine-induced immune function, the data will be used to describe the longitudinal changes in immune function with and without vaccine, in subjects to do and do not reactivate CMV. With this amendment, we will similarly describe the longitudinal changes in patients receiving Prevymis as prophylaxis. The combined analysis T-cell data using all subjects will be stratified by Prevymis, and the original power calculation remains relevant to this comparison.

With regard to the incidence of CMV events, the sample size of 48 individuals per arm was originally designed to detect a reduction in CMV reactivation from 40% to 15% at 100 days post-HCT, providing 90% power at one-sided 10% significance level (these are the typical operational characteristics in randomized Phase II trials [21]). The aim of detecting an overall benefit in all vaccinate patients has been abandoned after the interim analysis for futility, and continued enrollment is based on the new primary aims.

The comparison of CMV event rates across CMVPepVax arms for the new Prevymis cohort is motivated as a futility analysis, for informing a subsequent decision about further development of CMVPepVax. The Operating characteristics of that evaluation, analogous to the power of a hypothesis test, are described above, under Sample size.
Formal stopping rules for safety are embedded in the design of this study. Clinical data will be monitored as they accumulate, and investigation of CMVPepVax will be suspended for safety review if there is evidence of serious treatment-related AEs.
Monitoring of the Prevymis cohort will, as described above, be done conservatively as both a continued monitoring of a series of patients receiving CMVPepVax, and as monitoring of a new series of patients receiving CMVPepVax in combination with Prevymis.

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

(2) Severe acute GVHD (aGVHD, grade 3-4) will be monitored as every 12^{th} subject on the vaccine arm reaches the 100 day evaluation point. aGVHD will be scored using Keystone consensus criteria[22]. The trial will be interrupted at each respective center for safety review by the COH and external site DSMCs if 6 or more of the first 12 recipients, or 9 of 24, or 11 of 36, experience Grade 3-4 aGVHD. This would be a significant elevation from the COH/UMN historical benchmark of 15% of allogeneic HCT recipients with matched sibling donors [23]. These rules are determined to limit the overall aGVHD false alarm probability to 0.02 if vaccination does not increase risk [23].

(3) Any serious AEs (SAE, grade 3-4) related to the vaccination within 2 weeks from each vaccination will be reviewed by the PMT when each 12^{th} patient on the CMVPepVax arm passes the 2 week post-injection time for their second injection.

Table of Contents

SECTION PAGE

Experimental Design Schema 4
Protocol Synopsis 5
Table of Contents 13
List of Tables and Figures 15
1.0 Goals and Objectives (Scientific Aims) 18
2.0 Background 19
2.1 Introduction/Rationale for Development 19
2.2 Overview and Rationale of Study Design 21
2.3 Preclinical Studies 23
2.4 Human Studies 24
2.5 Interim analysis and rationale for continuation of the trial 35
3.0 Participant Eligibility Criteria 39
3.1 Pre-HCT Inclusion Criteria 39
3.2 Pre-HCT Exclusion Criteria 40
3.3 Participation of Special Populations 41
4.0 Participant Enrollment and Randomization 41
4.1 Pre-Enrollment Informed Consent and Screening Procedures 41
4.2 Participant registration 41
4.3 Randomization 42
4.4 Emergency De-Blinding Procedures 43
5.0 Treatment Program 43
5.1 Treatment Overview 43
5.2 Maintaining a blinded randomization 43
5.3 Vaccine Administration and Pre-Vaccination Prophylaxis 44
5.4 Assessments 44
5.5 Criteria for Completing/Discontinuing Study Participation 46
5.6 Follow-Up and Duration of Participation 46
5.7 Supportive Care, Other Concomitant Therapy, Prohibited Medications 47
6.0 Vaccine Administration Criteria 48
6.1 Day 28-Post-HCT Vaccine Administration Criteria 48
6.2 Day 56-Post-HCT Vaccine Administration Criteria 48
7.0 Adverse Events List and Reporting of Unanticipated Problems and Adverse EventS 49
7.1 Toxicities to CMVPepVax 49
7.2 Toxicities to the Placebo Vaccine 49
7.3 Definitions 49
7.4 Routine Reporting of Adverse Events by Site Investigators 51
7.5 Expedited Reporting of Unanticipated Problems and SAEs by Site Investigators 51
7.6 Reporting of Adverse events by the Study PI 52
8.0 Agent Information 54
8.1 CMVPepVax and Placebo Vaccine –information applicable to vaccine components 54
8.2 Tet-CMV, the synthetic peptide contained in CMVPepVax 55

8.3 PF-03512676, the adjuvant of CMVPepVax 56
8.4 Sodium acetate, the synthetic peptide diluent contained in CMVPepVax 56
8.5 Neut™ Sodium Bicarbonate Additive Solution for the Placebo Vaccine 57
8.6 Preparation of CMVPepVax 58
8.7 Preparation of Placebo Vaccine 59
9.0 Correlative/Special Studies 60
9.1 Immunogenicity testing 60
10.0 Study Calendar 62
11.0 Endpoint Evaluation Criteria/Measurement of Effect 64
12.0 Statistical Considerations 65
12.1 Study Design 65
12.2 Randomization 66
12.3 Sample Size Accrual Rate 66
12.4 Data Analysis Plan 67
12.5 Safety Monitoring 68
13.0 Data Handling, Data Management, Record Keeping 68
13.1 Source Documents 68
13.2 Data Capture Methods and Management 69
13.3 Case Report Forms/Data Submission Schedule 69
13.4 Regulatory Records 70
14.0 Adherence to the Protocol 70
15.0 Study Oversight, Quality Assurance, and Data & Safety Monitoring 71
15.1 Site Principal Investigator 71
15.2 Study Principal Investigator 71
15.3 Protocol Management Team (PMT) 72
15.4 Monitoring 72
15.5 Quality Assurance 72
15.6 City of Hope Data and Safety Monitoring Committee 72
16.0 Ethical and Regulatory Considerations 73
16.1 Ethical Standard 73
16.2 Regulatory Compliance 73
16.3 Institutional Review Board 74
16.4 Informed Consent 74
16.5 Women, Minorities, Children, HIV-Positive Individuals (Special Populations) 74
16.6 Participant Confidentiality 75
16.7 Conflict of Interest 76
16.8 Financial Obligations, Compensation, and Reimbursement of Participants 76
16.9 Publication/Data Sharing 76
17.0 References 78
Appendix A: List of Supplemental Materials 83
Appendix B: Acute GVHD Staging 84
Appendix C: Chronic GVHD Grading 85
Appendix D: Karnofsky Performance Scale 86

Appendix E: Reagents, Equipment and Supplies Necessary for Vaccine Preparation 87
Appendix F: PREPARATION OF CMVPEPVAX USING INITIAL LOT (#L0402009) OF TET-CMV 88
Appendix G: SAE/UP Reporting Coversheet 90
Appendix H: Registration Coversheet 91

List of Tables and Figures

Figure 2.3.1 Potency of GMP-grade Tet-CMV with and without PF03152676 adjuvant in HLA A*0201 HHDII transgenic mice 24
Table 2.4.1a Adverse events at least possibly related to CMV peptide (Tet-CMV and PADRE-CMV) with or without adjuvant in healthy volunteers 25
Table 2.4.1b Adverse events at least possibly related to CMVPepVax in CMV positive healthy volunteers vaccinated with 2.5 mg Tet-CMV peptide with adjuvant (CMVPepVax) 26
Figure 2.4.1a Longitudinal profiles of dsDNA autoantibody levels in sera of healthy volunteers vaccinated with CMVPepVax or CMV peptide without adjuvant. 27
Figure 2.4.1b Longitudinal profiles of *ex vivo* $pp65_{495\text{-}503}$ $tetramer^{+}$ $CD8^{+}$ T cell levels in response to CMV peptide with and without adjuvant, grouped by strength of pre-vaccination *ex vivo* $pp65_{495\text{-}503}$ $tetramer^{+}$ $CD8^{+}$ T cell level 28
Figure 2.4.1c IVS $pp65_{495\text{-}503}$ or the $HIVgag_{77\text{-}85}$ CD8+ T-cells as a percentage of all IVS CD8+ T-cells in healthy volunteers vaccinated with CMV peptide + PF-03512676 29
Table 2.4.2 List of SAEs in CMVPepVax vaccinated HCT recipients (N=17) 30
Figure 2.4.2a Longitudinal profiles of dsDNA IgG autoantibody levels (AAU/ml) in sera of HCT recipients vaccinated with CMVPepVax 31
Figure 2.4.2b aGVHD and cGVHD in HCT recipients vaccinated with CMVPepVax and control HCT patients ..32
Figure 2.4.2c CMV-$pp65_{495\text{-}503}$ pentamer CD8+ T cell amplification in HCT recipients vaccinated with CMVPepVax 33
Figure 2.4.2d CD8 T cells binding to CMV pp65 and HIV gag (control) MHC class I pentamers in a representative HCT recipient vaccinated with CMVPepVax (UPN8) 33
Figure 2.4.2d Kinetics of CD8+ T cells/ul binding to CMV- pp65 and HIV gag (control) MHC class I pentamers in a representative HCT recipient vaccinated with CMVPepVax (UPN 14) 34
Table 2.5 Interim Analysis 29
Table 10.0 Study Activity Calendar 62
Table 13.3 Data Submission Schedule 69

ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse Event
aGVHD	Acute Graft Versus Host Disease
BDP	Biopharmaceutical Development Program NCI-Frederick, MD
CBC	Complete Blood Count
cGVHD	Chronic Graft Versus Host Disease
cGMP	Current Good Manufacturing Practice
CLIA	Clinical Laboratory Improvement Amendments

CMV	Cytomegalovirus
CMVPepVax	Tet-CMV co-injected with PF-03512676
COH	City of Hope
CRA/CRC	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
FDA	Food and Drug Administration
FOS	Foscarnet
GCP	Good Clinical Practice
GCV	Ganciclovir
GVHD	Graft versus host disease
HBV	Hepatitis B virus
HCT	Hematopoietic Stem Cell Transplant
HCT-R+	CMV positive HCT recipients
HCV	Hepatitis C virus
HHV6	Human herpes virus 6
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HSV	Herpes simplex virus
HVTN/DAIDS	HIV Vaccine Trials Network/Division of AIDS
IB	Investigator Brochure
IDS	Investigational Drug Service
ICF	Informed Consent Form
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
IVS	*In vitro* stimulation
MRD	Matched Related Donor
MUD	Matched Unrelated Donor
NCI	National Cancer Institute
NRM	Non-relapse mortality
OIDRA	Office of IND Development and Regulatory Affairs
PBMC	Peripheral Blood Mononuclear Cells
PD-1	Program death receptor-1
PF-03512676	Pfizer synthetic single stranded CpG DNA adjuvant
PI	Principal Investigator
R^+	CMV positive HCT recipients
PMT	Protocol Monitoring Team
SAE	Serious Adverse Event
SAIC	Science Applications International Corporation
SC	Subcutaneous
SOC	Standard Of Care
Tet-CMV	Tetanus-CMV
Tg	Transgenic
T_H	T helper
TLR9()	Toll-like receptor 9
TTL	Translational Therapy Laboratory
UMN	University of Minnesota Medical Center
VAL	Valganciclovir

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

Primary Objectives:

For the entire cohort (n=97):
1) To determine if CMVPepVax increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated compared to placebo treated HLA A*0201, CMV seropositive HCT-recipients,

For the Prevymis combination cohort (n=36)

1) To provide a preliminary evaluation of the incidence of CMV reactivation between day 56 and day 180 in patients who receive standard Prevymis prophylaxis (from day 14 through day 100), comparable to the evaluation of an expansion cohort in a pilot study, or the futility stage of a phase II trial

2) To determine if CMVPepVax increases levels, function and kinetics of CMV-specific T cell immunity in vaccinated HCT patients who receive standard Prevymis prophylaxis.

Secondary Objectives:

1. To determine, within the constraints of a pilot cohort, if CMVPepVax reduces the frequency of CMV events alone or in combination with Prevymis defined as reactivation or CMV disease in HLA A*0201 allogeneic HCT-R+. A CMV event encompasses any detection of CMV by either qPCR (termed “reactivation”: DNAemia ≥500 gc/ml or by tissue histology (end-organ disease).
2. To evaluate the safety and tolerability of CMVPepVax by assessing the following: non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and grade 3-4 AEs (CTCAE 4.0) probably or definitely related to the vaccination within 2 weeks from each vaccination.
3. To characterize CMV reactivation and CMV disease in recipients of CMVPepVax compared to placebo by assessing time to viremia (defined as number of days from transplantation to the date of ≥500 CMV gc/mL), duration of viremia, recurrence of viremia, incidence of late CMV viremia/disease (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia, cumulative number of CMV specific antiviral treatment days. To evaluate the impact of CMVPepVax on transplant related outcomes by assessing the incidence of acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, non-CMV infections.
4. To determine whether vaccination induces adaptive NK cell population changes, and increase in the highly cytotoxic memory NKG2C+ NK cells,
5. To determine the impact of CMVPepVax on CMV immune reconstitution in patients who undergo treatment with antiviral agent Prevymis
6. To explore GVHD biomarkers and compare between the vaccine and placebo groups,
7. To characterize CMV reactivation after day 180

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Human CMV is a highly prevalent, globally-occurring infection that rarely elicits disease in healthy immunocompetent hosts. The human immune system is unable to clear CMV infection and latency, but mounts a spirited immune-defense targeting multiple immune-evasion genes encoded by this double-stranded DNA β-herpes virus. Encoding around 165 genes, CMV is among the largest and most complex of known viruses [9]. The size of the CMV-specific cellular immune response is the most striking aspect of the dynamic, life-long interaction between the host and CMV. CMV-specific T cells are essential to restrain CMV viral replication and prevent disease, though do not eliminate the virus or preclude transmission.

Significant suppression of host antiviral immunity can alter the life-or-death immune surveillance homeostasis, allowing either CMV reactivation to become detectable or primary infection to cause clinical symptoms. Uncontrolled viral replication and dissemination results in the development of life-threatening end-organ damage (CMV disease) [24-26]. CMV infection is the cause of major complications and significant morbidity in the recovery of immune-compromised recipients both at early and late times post- HCT [3, 5, 6]. HCT patients are vulnerable to herpes virus infections, including CMV, as a result of immunosuppression associated with treatment strategies aimed at preventing rejection or GVHD [27-29]. Pharmacologic agents used to limit virus replication, such as, GCV or its oral form VAL, and FOS have become the methods of choice for prophylaxis of CMV infection [3, 30]. Despite this, CMV remains an important cause of mortality after HCT diminishing the full curative potential of this successful cancer therapy [10, 31]. Furthermore, anti-viral chemotherapy has major side effects, including nephrotoxicity, neutropenia, and delayed immune reconstitution which exposes HCT recipients to other opportunistic viral, bacterial and fungal infections [6, 32]. For example, the use of GCV/VAL is associated with a higher proportion of recipients becoming neutropenic and increased numbers of concomitant fatal infections [6, 32]. As GCV/VAL therapy has become ubiquitous in practice, delayed onset of CMV-pneumonia (interstitial pneumonitis, IP) is more frequent which suggests that GCV/VAL impairs immunologic reconstitution [33, 34]. When antivirals are stopped or when virus resistance occurs, the same disease symptoms appear; only frame shifted to ~180 days post-HCT [5, 35-37]. Thus, different strategies to control CMV are eagerly sought, since antivirals are at best a stop-gap measure, and their use does not address the major risks of late-onset CMV-IP including early CMV reactivation and failure to reconstitute CMV-specific immunity [31]. Additionally costs associated with the usage of antivirals are significant, with one single course of GCV/VAL reaching $25,000.

Prevymis has been approved by the FDA for all CMV seropositive HCT recipients to minimize the chance of CMV disease development and the use of Prevymis for this purpose is now adopted into the HCT SOP. In a separate cohort we are intending to study the highly novel combination of Prevymis and CMVPepVax vaccine developed by COH. This combination has no overlapping toxicities, and is potentially synergistic, or at least should possess additive protective effect for CMV infection. This is particularly promising since CMV-specific T cell immune reconstitution has not been described in patients receiving Prevymis, and late CMV reactivation after stopping Prevymis on day 100 remains a major clinical problem. This problem can be potentially overcome by the combination of Prevymis and CMVPepVax.

Substituting or combining antivirals with a vaccine that harnesses the abundant native immune response to CMV may improve outcomes for HCT recipients. In particular, a CMV vaccine that confers protective immunity early post-transplant, until normal immunocompetence is re-established in the HCT recipient (6 months or earlier post-HCT) may reduce CMV morbidity and the use of antivirals [12]. It has been shown that control of CMV infection is primarily associated with cellular immune responses [38]. The abundant tegument pp65 protein is a major contributor to shaping the T cell repertoire in CMV-

exposed individuals [39-41], and is a principal target for HLA Class I-restricted $CD8^+$ CTL [9]. CMV-infected cells express pp65 both early and late after infection, making it an appropriate vaccine target [42-45]. Early clinical studies showed that pp65 CTL development is necessary to overcome CMV disease in HCT recipients [2, 46, 47]. Adoptive transfer of pp65-specific CTL reduced CMV viremia after HCT in Phase I trials [48-52]. In patients who have measurable CMV viremia, expansion of pp65 specific $CD8^+$ T cells occurs, especially after viral reactivation [53, 54]. In agreement with those observations, studies showed that CMV reactivation and disease were detectable only in patients with low level of CMV-tetramer$^+$ $CD8^+$ T cells [10, 55]. Importantly, several reports have associated protection from CMV complications in HCT recipients with CMV-specific $CD8^+$ T cell levels (including pp65-specific T cells) between 7-10/µL [10, 13]. A vaccine that induces protective levels of pp65 CTL has the potential to be of therapeutic benefit to an HCT recipient by limiting CMV viremia or disease [9, 10, 43-45, 48, 52, 56].

Our group has identified a repertoire of CTL epitopes within the pp65 protein that can expand human pp65-specific memory CTLs *in vitro* [39, 40, 57]. The $pp65_{495-503}$ epitope restricted to the high frequency HLA A*0201 allele has been extensively characterized [39-41]. Usage of the $pp65_{495-503}$ CTL epitope is conserved with limited sequence variation among viral isolates [40, 57, 58]. Additionally, using HLA-restricted CTL epitopes to develop a non-infectious subunit CMV vaccine can eliminate the safety concerns for HCT recipients of live-attenuated CMV or recombinant live viral vaccines, while avoiding the many CMV-encoded products involved in immune-evasion [43, 59, 60].

We discovered that covalently linking the pp65 CTL epitope to native tetanus epitopes by solid phase synthesis, dramatically enhanced the immunogenicity of epitope vaccines [7, 8]. In HLA A*0201 Tg mice, two candidate vaccine peptides containing the HLA A*0201 $pp65_{495-503}$ $CD8^+$ T cell epitope fused to universal T_H epitopes (either the synthetic PADRE or a natural Tetanus sequence) showed favorable immunogenicity profiles [7, 8, 61, 62]. The vaccine peptides were named PADRE-CMV and Tet-CMV, respectively [8]. Co-injection with PF-03512676 adjuvant (a synthetic single stranded CpG DNA with immunostimulatory activity [63]) further augmented their activity, providing a means to lower vaccine dosage [7, 64].

cGMP-grade PADRE-CMV and Tet-CMV, with or without PF-03512676 adjuvant were clinically evaluated for safety and immunogenicity. The Phase Ib dose-escalation clinical trial (COH IRB 03121, NCT00722839@www.clinicaltrials.gov.) was conducted in HLA A*0201 healthy adults and indicated that the CMV peptides co-injected with PF-03512676 were safe and immunogenic. In particular, 2.5 mg Tet-CMV + PF-03512676 adjuvant in a final 1.0 ml volume, administered in the upper arm by SC route had a favorable safety profile and led to substantial expansion of $pp65_{495-503}$ T cells after 2 vaccine injections. These data supported further evaluation in the HCT setting [8] of this formulation of Tet-CMV combined with PF-03512676, which was renamed CMVPepVax.

In HCT recipients with nascent hematopoietic reconstitution and prolonged $CD4^+$ T cell deficit, stimulation of TLR9 by PF-03512676 may substitute for the requirement of CD4-T_H to sustain immune responses [65]. TLR9 is implicated as an endosomal receptor for bacterial CpG DNA motifs exemplified by PF-03512676 [63, 66]. While immune-reconstitution can be limited in allo-HCT population, CMVPepVax has the potential for greater potency when injected into CMV^+ HCT recipients, since massive expansion of pp65-specific T cells have been shown in HCT patients under the conditions of CMV infection [10, 52]. Based on these findings, we performed an evaluation of safety and immunogenicity of two injections of CMVPepVax, in HLA A*0201 CMV-positive adult recipients of an HLA-matched allogeneic HCT for hematologic malignancies (COH IRB 12022 NCT01588015@www.clinicaltrials.gov.) which as of January 15th, 2015 has completed enrollment.

In this open label Phase Ib trial, 36 allogenic HCT CMV positive recipients (R^+) are randomized either to the CMVPepVax vaccine arm or the observational arm. HCT recipients in the vaccine arm are injected twice on days 28 and 56 post-HCT with CMVPepVax, during the critical period in which primary CMV

reactivation most predictably occurs (~day 40-100) [35, 67]. CMVPepVax has been safely administered to 18 HLA A*0201 R^+ patients. Preliminary results indicate excellent tolerability, safety and substantial increases of CMV $pp65_{495-503}$ CD8 T cells, without increased incidence of GVHD, in patients immunized with CMVPepVax. In particular, we observe a reduction in CMV reactivation and a significantly lower incidence of cGVHD in the CMVPepVax arm compared to the observational arm. Interestingly, all CMVPepVax vaccinated recipients continue to remain cancer free, while 3 observational patients experienced relapse of their hematological malignancy. Thus, we have safely treated HCT-R^+ with CMVPepVax, but a crucial unattained objective is to assess vaccine protection against CMV reactivation and disease by the vaccine itself or in combination with Prevymis administration. Consequently, there is a strong rationale to test CMVPepVax using a randomized, blinded and placebo controlled Phase II efficacy trial. This proposed clinical study is designed to be a multicenter trial. Moreover, NCI NExT is providing a new lot of vaccine to support this multi-center Phase II study.

2.2 Overview and Rationale of Study Design

This is a randomized, blinded and placebo controlled Phase II efficacy trial which will be conducted as a multicenter clinical trial at COH and collaborating, external sites. This study has been designed to have sufficient statistical power for testing clinically significant endpoints. In particular, the goal of this Phase II clinical trial is to assess efficacy of CMVPepVax by itself and in combination with Prevymis administration, in protecting against CMV reactivation and disease in HLA A*0201 HLA-matched allogeneic (related or unrelated donor) HCT R^+ (CMV positive HCT recipients) who are at significant risk for CMV complications [13]. The primary hypothesis to be tested is that immunizing HLA A*0201 allogeneic HCT-R+ with CMVPepVax will provide superior protective benefit compared to a placebo. The secondary hypothesis is that CMVPepVax immunizations will induce protective levels of CMV cellular immunity in HCT-R+ [63, 68, 69]. It is anticipated that the trial will provide definitive data on vaccine-induced protective immunity against CMV reactivation and disease, in allogeneic HCT recipients [10].

Participants must be planned recipients of a first 8/8 HLA-matched [70-73] allogeneic HCT for hematological malignancies, be HLA A*0201, CMV-positive, age 18-75 (chronologic age is no longer considered a contraindication for HCT [74, 75]), and willing to be monitored for 12 months following HCT. Exclusion criteria include receiving T cell depleted HCT, autoimmune disease, HIV, HCV and HBV positivity. Excluded diagnosis are aplastic anemia and multiple myeloma. Screening, enrollment and informed consent procedures will occur prior to the HCT procedure, as the prospective participant is likely better able to fully consider the study and provide an informed decision as the prospective participant's status is more stable during this period. Vaccine administration criteria will be assessed on the day of planned vaccine administration (Day 28 post-HCT). The Day-28-Post-HCT criteria exclude participants who, post HCT, have experienced relapse of disease, have experienced CMV viremia or end organ disease, have received anti-viral treatment, have received high doses of steroids within 7 days of planned vaccine administration, or are experiencing ongoing grade 3 toxicities.

This phase II efficacy trial will enroll and vaccinate 97 HLA A*0201 allogeneic HCT-R^+, randomized 1:1 to either the CMVPepVax arm (N=48), or to the placebo arm (N=48). The first 61 subjects did not receive Prevymis, and were closed to accrual after the interim analysis, while the next cohort consisting of 36 of these randomized subjects will receive Prevymis treatment in addition to CMVPepVax or placebo. The resulting data will be stratified by agent administration (vaccine or placebo) and Prevymis administration (Prevymis or no Prevymis). Thus, the study will be blinded for CMVPepVax and open label for Prevymis. Because approximately 10-15% of enrolled participants may fail to meet post-HCT vaccine administration criteria, the total study accrual is expected to reach 106-115 participants. All patients in the new cohort (36 patients, 18 vs 18) will receive Prevymis as a standard of care. The size of the new cohort is based on the budgetary constraints of the original study plan.

A computer-generated 1:1 randomization stratified by donor CMV serostatus will assign participants to the CMVPepVax or placebo arms. The registrar located at the COH Data Coordinating Center will contact either COH or the external sites to provide treatment assignment to the site pharmacists, who are unmasked to treatment-group allocation. Because visual inspection of the vaccine at the time of administration may reveal the arm to which a participant has been assigned, persons administering the vaccine will receive training about how not to reveal the treatment arm to the participant and colleagues. All study team members will receive orientation about the importance of maintaining a blinded randomization status.

Participants meeting vaccine administration criteria will receive injections of either CMVPepVax or placebo on days 28 and 56 post-HCT, during the critical period in which primary CMV reactivation most predictably occurs (~day 40-100)[35, 67]. Both the CMVPepVax and placebo agents are administered at a final 1.0 ml volume, in the upper arm by subcutaneous route.

CMVPepVax vaccine formulation and dosage (approx. 2.5 mg Tet-CMV + 1.08 mg PF-03512676 adjuvant diluted in sodium acetate) were chosen based on the data from the Phase Ib trial in healthy adults (COH IRB 03121), indicating mild reactogenicity and a favorable safety profile for this specific formulation when up to 4 SC injections were administered to healthy volunteers [8]. Preliminary results from the completed Phase Ib trial (COH IRB 12022) in HLA A*0201 MRD and MUD HCT R^+ indicate excellent tolerability, safety and substantial increases of CMV $pp65_{495\text{-}503}$ CD8 T cells in patients immunized with CMVPepVax using this formulation and dosage on Day 28 and Day 56 post-HCT, the same schedule to be used in this study.

CMVPepVax administration provides a dose of 2.48mg Tet-CMV Peptide + 1.06mg PF-03512676 when the vaccine is prepared using the currently available Tet-CMV (LOT# L0402009, 11mg/1.1mL vial).

CMVPepVax administration provides a dose of 2.64mg Tet-CMV Peptide + 1.08mg PF-03512676 when the vaccine is prepared using the new lot of Tet-CMV (LOT#L1501005, 3.3 mg/ml, fill volume 1.2mL).

Participants in the placebo arm will be vaccinated with an isotonic solution of cGMP-grade solution of sodium acetate/ Neut™ (4% Sodium Bicarbonate Additive Solution), the diluent used in the Phase Ib dose-escalation clinical trial (COH IRB 03121, NCT00722839@www.clinicaltrials.gov.) for Tet-CMV without PF-03512676 adjuvant. Participants who do not receive the first (Day 28) vaccine will be replaced and removed from the study. All participants who receive the first vaccine will be followed and assessed through Day 365 post-HCT, except for participants who demonstrated disease relapse. Participants with relapsed disease will be in need of starting other therapies to control their disease, and therefore will be followed for survival only through Day 365.

HCT recipients are heavily monitored for safety according to institutional standard of care practices, which recommend intense monitoring through the first 100 days following the HCT procedure. This study will depend on the standard of care (SOC) practices through Day 100 post-HCT of weekly or biweekly SOC assessments of CMV qPCR, clinical laboratory tests, engraftment assessment, GVHD assessment and physical exam, and, when clinically indicated, CMV disease assessment and disease relapse assessment; disease relapse is also routinely assessed at pre-determined time points per SOC. The procedures and clinical care provided to participants should not differ from that provided routinely to all post-HCT patients with the exception of restrictions on concomitant medications that might impact study endpoints such as prophylactic antiviral treatment, and T-cell depleting agents, and the administration of other vaccines whose prohibition is lifted after the second vaccination.

From Day 28 through Day 100, study visits will occur bi-weekly, to coincide with vaccine administration and research blood sample collection, however information relevant to study endpoints will be obtained from any clinic visit, not just the at the corresponding study clinic visit. After Day 100, an additional four

study visits will occur up to and including Day 365 to ensure periodic documentation and/or evaluation of clinical endpoints and for the collection of research blood samples.

Because data from the phase 1b study in HCT CMV-positive recipients demonstrated decreased disease relapse and chronic GVHD in CMVPepVax recipients, the study will investigate the impact of CMVPepVax on transplant related outcomes such as GVHD, disease relapse, mortality, and infection rate.

Rigorous stopping rules will be implemented, and will include three major safety endpoints; non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) acute GVHD (aGVHD), and grade 3-4 AEs (CTCAEv4) related to the vaccination within 2 weeks from each vaccination. There will be an interim futility analysis once the first 48 patients are evaluable for the frequency of CMV reactivation before day 100 (included in the primary endpoint). If the observed CMV viremia-rate in the CMVPepVax arm is higher than on the placebo arm, the study will be suspended for NCI and DSMC review [14]. The loss of power of invoking a stopping rule at 50% of accrual is less than 0.02[14].

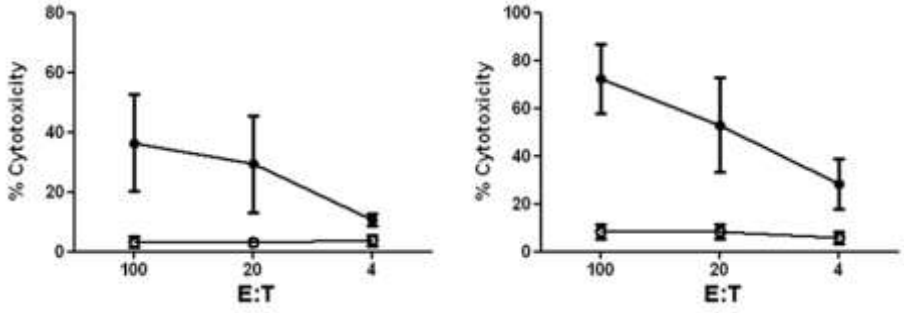
CMV-specific immunogenicity (included in the secondary endpoints) will be evaluated in all vaccinated participants from all arms (N=97) every 2 weeks from day 28 until day 100 post-HCT, and afterward on days 140, 180, 270, 365. Immunologic studies will include monitoring levels of CD8+ T cells binding to pp65 A2-CMV-dextramers®, combined with immunophenotyping studies [15, 16]. The phenotypic ratios of CMV-specific T cells will be related to improvement in control of CMV viremia. Based on the encouraging results of the pilot Phase Ib (COH IRB 12022), levels of pp65-specific T cells associated with protection from CMV viremia and disease are anticipated to be detected in a significantly higher proportion of vaccine recipients compared to those enrolled in the placebo arm [10]. Additional correlative immunogenicity studies (included in the secondary endpoints) will include measuring levels of highly cytotoxic memory NKG2C+ NK cells, linked to CMV reactivation, critical for CMV adaptive immune response and potentially linked to relapse reduction [17]. In the phase Ib trial of CMVPepVax in HCT recipients (COH IRB 12022) CMVPepVax administration was associated with reduced risk of chronic GVHD while there was no increase in acute GVHD. To further investigate potential immunologic impacts of CMVPepVax on GVHD, various GVHD biomarkers will be evaluated including various cytokines and hepatocyte growth factor for systemic GVHD [76, 77], elafin for skin GVHD [78], regenerating islet-derived 3α (REG3α) for gastrointestinal GVHD [79], suppression of tumorigenicity 2 (ST2) for steroid-refractoriness 5 [80], and CXCL9 [81, 82]and B cell–activating factor (BAFF) for chronic GVHD [81, 82]. The levels of these GVHD biomarkers will be compared between the vaccine group and placebo group, as well as between GVHD+ and – groups.

2.3 Preclinical Studies

2.3.1 cGMP-grade Tet-CMV ± PF-03512676 adjuvant

Tet-CMV ± PF-03512676 adjuvant (CMVPepVax = 2.5 mg Tet-CMV + 1 mg PF-03512676) has been extensively tested in Tg mouse models adults [7]. The cGMP-grade CMV vaccine products were evaluated in HLA A*0201 HHDII Tg mice, and potency results were confirmed, as illustrated in Figure 2.3.1 [8].

Figure 2.3.1 Potency of GMP-grade Tet-CMV with and without PF03152676 adjuvant in HLA A*0201 HHDII transgenic mice



*HLA A*0201 HHDII transgenic mice were immunized with 100 nmoles of Tet-CMV (left) and Tet-CMV + PF03512676 (right). The plots show percentages of cytotoxicity at different effector to target rations (E:T) detected in a 4-h chromium release assay against either relevant ($pp65_{495-503}$, black circles) or control ($p53_{149-157}$, white circles) target cells. Data in each plot are representative of at least 6 immunized mice.*

2.3.2 Animal Toxicology

Pre-clinical toxicology studies in rats revealed little to no toxicity of the cGMP-grade CMV peptides at all investigated dosages (from Southern Research Institute, Protocol 11200.11.01).

2.4 Human Studies

The proposed Phase II clinical trial in the HCT setting is supported by the promising safety data and immunogenicity profiles obtained using this vaccine formulation first in a clinical study performed in a cohort of healthy volunteers (IRB 03121), and subsequently in a single center pilot Phase Ib in HCT patients, that was completed at COH (IRB 12022).

In particular, based on IRB (03121) and FDA (IND, BB-13124) approval, cGMP-grade PADRE-CMV and Tet-CMV, with or without PF-03512676 adjuvant were clinically evaluated for safety and immunogenicity (Phase Ib trial) in healthy adults, expressing the HLA A*0201 MHC Class I allele sequence. The healthy adult study indicated that CMVPepVax was safe and induced robust expansion of CMV-pp65 CD8 T cells. These data supported further evaluation of CMVPepVax in HLA A*0201 HCT recipients[8]. The completed Phase Ib pilot trial (IRB 12022) is designed to evaluate safety of administering CMVPepVax to HLA A*0201 MRD or MUD R^+ at risk for CMV complications [13]. The published results have shown reliable safety, reduced CMV reactivation and cGVHD in HCT recipients injected with CMVPepVax compared to HCT recipients in the observation arm .

2.4.1 Phase Ib clinical trial in healthy adults (COH IRB 03121)

The Phase Ib safety and immunogenicity clinical trial, performed at COH in healthy individuals was a non-randomized, open label, dose escalating study (NCT00722839@www.clinicaltrials.gov)[8]. Healthy male and female volunteers, ≥18 and ≤55 years old, CMV-seropositive or seronegative, and molecularly subtyped as HLA A*0201-positive were enrolled after signing informed consent. Participants were eligible unless they had ≥1 of the following exclusion criteria: abnormal serum chemistry and blood count; hepatitis B or C positive; immunodeficiency, including HIV; taking daily medications for chronic illness, surgery within 6 months of vaccination requiring general anesthesia; known cardiac disease including patients being treated for hypertension and high cholesterol; positive urine pregnancy test/planning to become pregnant within 6 months from vaccination; immunization with other vaccines within 1 month of the study period; participation in a CMV immunotherapy trial in the previous 6 months; history of cancer, depression, allergic diatheses, frequent migraines.

The safety (primary endpoint) and immunogenicity (secondary endpoint) evaluation of CMV peptides (Tet-CMV, and PADRE-CMV) was performed separately in two concomitant stages: Tet-CMV or PADRE-CMV peptide alone and Tet-CMV or PADRE-CMV peptide co-injected with 1 mg PF-03512676 adjuvant [83]. The vaccine dose escalation (0.5, 2.5, 10 mg vaccine) was based on previous peptide vaccine studies[84]. A cGMP-grade sodium acetate solution was used as peptide diluent. Tet-CMV peptide formulation was comprised of 1 ml of peptide solution and 0.1 ml sodium bicarbonate USP to adjust pH; Tet-CMV peptide with adjuvant was comprised of 1.0 ml of peptide solution and 0.1 ml PF-03512676 (1 mg). The final 1.0 ml injection volume was administered SC in the upper arm. To assess safety of multiple injections, booster doses of vaccine ± PF-03512676 (1 mg) were given at day 21, 42, 63. Six volunteers were allotted to each dose level and the vaccine was administered by SC injection in the upper arm.

Safety in Healthy Adults: Adverse Events

The peptide vaccines were safe and tolerated in most subjects, though addition of the PF-03512676 (1 mg) adjuvant increased reactogenicity (Table 2.4.1a). Most common AEs included mild to moderate cutaneous reactions at the injection site and systemic flu-like symptoms. The duration of related Grade 1 and 2 AEs ranged from 1-2 days. Grade 3 AEs included marked malaise, 39.4–40.5°C fever, urticaria and were resolved with non-prescription analgesics (acetaminophen and antihistamine respectively), within 8 days. Of participants receiving Tet-CMV + PF-03512676 2 (15.3%) subjects experienced Grade 2 and 1 (7.7%) Grade 3 AEs. No association was found between severity or grade of AEs and pp65-specific immunity.

Table 2.4.1a Adverse events at least possibly related to CMV peptide (Tet-CMV and PADRE-CMV) with or without adjuvant in healthy volunteers

	PADRE-CMV + PF03512676 (N=19)			Tet-CMV + PF03512676 (N=13)			PADRE-CMV (N=19)			Tet-CMV (N=12)			Total vaccinated (N=63)
AE	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**% Total**
Local													
Pain	1	0	0	1	0	0	2	1	0	0	0	0	8%
Pruritus	1	0	0	1	0	0	0	0	0	0	0	0	3%
Erythema	0	1	0	3	0	0	1	0	0	0	0	0	8%
allergic reaction	2	2	1	1	1	1	2	0	0	0	0	1	17%
Systemic													
Malaise	7	3	1	2	0	1	3	1	0	3	1	0	35%
Fever	2	0	1	2	1	0	0	0	0	2	0	0	13%
Chills	4	1	0	4	0	0	0	0	0	0	0	0	14%
Myalgia	2	2	0	2	0	0	0	0	0	3	0	0	14%
Arthralgia	3	0	0	0	0	1	1	0	0	1	0	0	10%
Nausea	2	0	0	0	1	0	0	0	0	0	0	0	5%
Vomiting	2	0	0	0	1	0	0	0	0	0	0	0	5%
Headache	0	2	0	0	1	0	2	0	0	2	0	0	11%

Reactogenicity to the synthetic immunogens in all vaccinees regardless of dosage of Tet-CMV. AEs are graded (top grey row) according to the HVTN/DAIDS intensityscale: Grade 1: Mild, Grade 2: Moderate, Grade 3: Severe, Grade 4: Life-threatening. The table shows the number of volunteers who experienced Grade 1, 2, or 3 AEs. No toxicity Grade 4 AEs were reported. N=number of vaccinated subjects. Last column shows the percentages of the post-vaccination related AEs in the total vaccinated population. AEs that were considered unrelated or unlikely to be related to the investigational agent are not provided.

In particular, in the cohort immunized with 2.5 mg Tet-CMV + 1 mg PF-03512676 adjuvant (the vaccine formulation used in HCT recipients and re-named CMVPepVax) a single Grade 2 AE (38.7–39.3°C fever) resolved in 1 day was reported for one vaccinated volunteer (Table 2.4.1b).

Table 2.4.1b Adverse events at least possibly related to CMVPepVax in CMV positive healthy volunteers vaccinated with 2.5 mg Tet-CMV peptide with adjuvant (CMVPepVax)

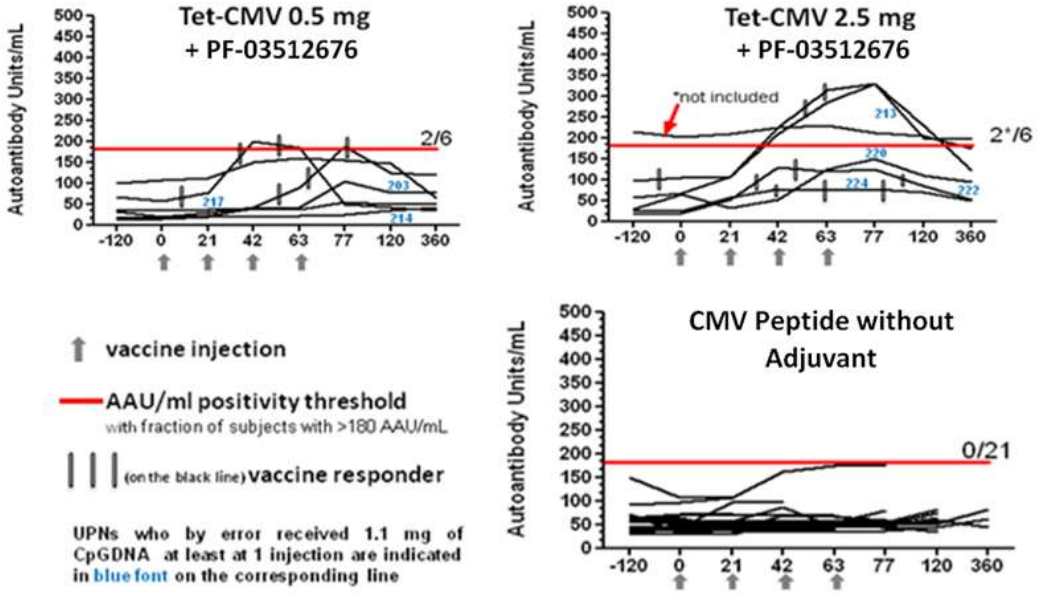
2.5 mg Tet-CMV + 1 mg PF03512676	AEs, relation: possible/probable/definite
CMV +ve UPN 220	none
CMV +ve UPN 213	none
CMV +ve UPN 222	**Grade 1**: malaise/fever/headache
CMV +ve UPN 224	none
CMV +ve UPN 228	**Grade 1**: malaise/fever/cutaneous reaction
CMV +ve UPN 226	**Grade 2**: fever. **Grade 1**: myalgia/cutaneous reaction

AE grades for the cohort of CMV positive (+ve) volunteers vaccinated SC four times with 2.5 mg Tet-CMV peptide vaccine + 1 mg PF03512676 (with the exception of UPN 228 who received 3 injections). For AE Grade definition see Table 2.4.1a. Only AEs that were considered possibly, probably or definitely related to the investigational agent are provided (as reported on the top of the right column).

Safety in Healthy Adults: Analysis of Double Stranded DNA Autoantibodies

Sera from immunized volunteers were longitudinally evaluated during the whole study observation period (from day -120 to ~1 year after the 1st immunization), for levels of double stranded (ds) DNA autoantibodies. These analyses were performed since mouse models of systemic autoimmune diseases have indicated involvement of TLRs in the generation of autoreactive immune responses[85]. The semi-quantitative detection of human dsDNA IgG autoantibodies in serum was performed using the dsDNA ELISA kit system, by Wampole Laboratories (Princeton, NJ) at COH General Clinic Research Centre. As shown in Figure 2.4.1a (lowest plot), no individuals (N=21) vaccinated with the CMV peptides only showed levels of dsDNA autoantibodies above the positivity threshold (>180 AAU/ml). In contrast, 66.7% (total N=18, not shown) and 36.4% (total N=11, Figure 2.4.1a top plots) of the healthy volunteers vaccinated respectively with Tet-CMV + PF-03512676 adjuvant demonstrated levels of dsDNA autoantibodies transiently raised above the positivity threshold following vaccination; by day 360 these levels returned within the normal range. Importantly, none of the immunized volunteers co-injected with PF-03512676 CpGDNA had any evidence of developing autoimmune disease or ever reported autoimmunity-related pathologies, including UPN 228 (2.5 mg Tet-CMV + PF-03512676) who maintained the same levels of dsDNA autoantibodies slightly above positivity threshold, before and after vaccination.

Figure 2.4.1a Longitudinal profiles of dsDNA autoantibody levels in sera of healthy volunteers vaccinated with CMVPepVax or CMV peptide without adjuvant.



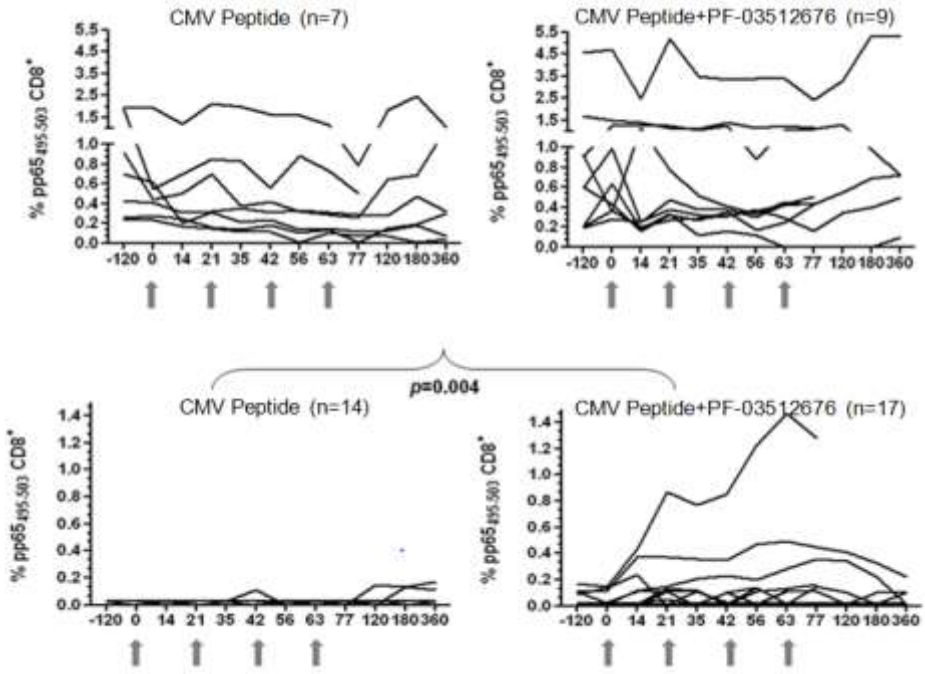
The addition of PF-03512676 adjuvant to CMV-Tet transiently raises the levels of dsDNA above the positivity threshold. The lower plot summarizes all results from vaccinees who received CMV-peptide (PADRE or CMV-Tet) without adjuvant. Numbers on the threshold red line indicate the fraction of immunized subjects with AAU/mL levels above positivity threshold.

Immunogenicity of CMVPepVax in Healthy Adults

The induction of post-vaccination *ex vivo* immune responses was monitored by flow cytometry analyses, using FACSCanto™ with FACSDiva software (BD Biosciences)[7]. Positive post-vaccination responses, defined as a >3 fold increase in either MHC class I $pp65_{495\text{-}503}$ tetramer binding or IFN-γ expression by $CD8^+$ T cells compared to baseline were exclusively detected in volunteers who received the vaccine co-administered with PF-03512676 (Figure 2.4.1b)[44].

Comparing the longitudinal profiles of *ex vivo* $pp65_{495\text{-}503}$ $tetramer^+$ $CD8^+$ T cell levels, a striking difference (Figure 2.4.1b; p=0.004, Wilcoxon rank-sum test comparing post-vaccination averages between d14-77) was noted in immunized volunteers whose pre-vaccination $pp65_{495\text{-}503}$ tetramer $CD8^+$ T cell levels were minimal-low (<0.2%, referred as $CMV^{+\ <0.2\%}$). For these CMV $pp65_{495\text{-}503}$ -immune-negative volunteers, recipients of the CMV peptide without adjuvant did not elicit a tetramer level increase during the whole observation period which included repeated vaccinations (Figure 2.4.1b, left bottom plot), whereas recipients of the CMV peptide vaccine with adjuvant frequently demonstrated increases in $pp65_{495\text{-}503}$ $CD8^+$ T cells after each vaccination (Figure 2.4.1b, right bottom plot).

Figure 2.4.1b Longitudinal profiles of *ex vivo* $pp65_{495\text{-}503}$ tetramer^{+} CD8^{+} T cell levels in response to CMV peptide with and without adjuvant, grouped by strength of pre-vaccination *ex vivo* $pp65_{495\text{-}503}$ tetramer^{+} CD8^{+} T cell level



Longitudinal profiles of ex-vivo $pp65_{495\text{-}503}$ specific tetramer binding (expressed in percentages) for recipients of CMV peptide alone and CMV peptide with PF-03512676. Upper plots: CMV-seropositives who had at baseline ≥ 0.2% $pp65_{495\text{-}503}$ tetramer binding. Lowev plots: baseline CMV+<0.2% $pp65_{495\text{-}503}$ tetramer binding. The p value indicates the difference between CMV peptide vaccinees with and without adjuvant in CMV+<0.2% subjects calculated by Wilcoxon rank-sum test. comparing post-vaccination averages between day 14-77.

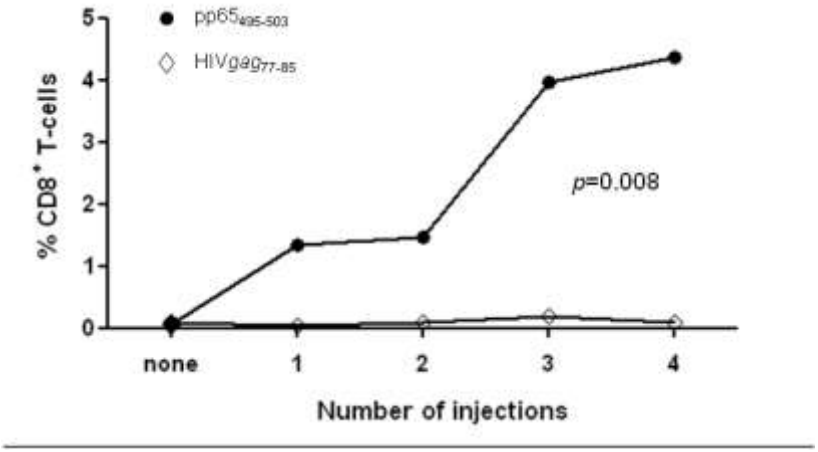
In all responders, *ex vivo* CMV responses were still detectable at day 77 (Figure 2.4.1b, right bottom plot). These results suggest that PF-03512676 adjuvant contributed to stimulating CMV vaccine responses, though its effect varied in the vaccinated CMV$^{+<0.2\%}$ population. Limited variation in $pp65_{495\text{-}503}$ tetramer levels were observed post-vaccination in the CMV-seropositive population (baseline ≥0.2% $pp65_{495\text{-}503}$ tetramer binding) when vaccinated with or without adjuvant (Figure 2.4.1b, top plots).

Though volunteers vaccinated with CMV peptide alone(without adjuvant) did not show *ex vivo* post-vaccination increases in $pp65_{495\text{-}503}$ CD8^{+} T cells, by using IVS we were able to detect priming of memory T cell responses (>10 fold expansions) in ~30% (4 of 14) of CMV$^{+<0.2\%}$ and ~40% (3 of 7) of CMV-seronegatives. In addition, $pp65_{495\text{-}503}$ CD8^{+} T cells could be consistently expanded in all 4 CMV-seronegatives and in 4 CMV-seropositive volunteers vaccinated with CMV peptide + PF-03512676 who failed to demonstrate *ex vivo* responses. Confirming the *ex vivo* data, the correlative IVS studies showed that usage of PF-03512676 adjuvant induced significantly higher (p=0.002, two-sided Fisher's exact test) post-vaccination responses when comparing responses of recipients CMV peptide + PF-03512676 to those of CMV peptide without adjuvant.

Stimulation strategies to evaluate vaccine immunogenicity in clinical trials can have limitations, due to length and conditions of the *in vitro* culture [44, 45]. However, breadth and consistency of post-IVS responses detected in recipients of the CMV peptide + PF-03512676 (Figure 2.4.1c) after brief exposure of PBMC to the $pp65_{495\text{-}503}$ peptide are promising. In fact, the vaccine elicited $pp65_{495\text{-}503}$ T cells could be significantly expanded upon exposure to CMV antigens in a viremic individual. In particular, the CMV peptide vaccines may have the potential for greater potency when injected into CMV positive HCT

recipients, as further massive expansion has been reported to occur under the natural stimulation of CMV infection[52]. The resulting $pp65_{495-503}$ T cell increase in HCT recipients can be critical to control CMV viremia [43-45]. In summary, the correlative IVS studies indicate that the CMV peptide vaccines + PF-03512676 are effective in consistently priming pp65-specific T cells.

Figure 2.4.1c IVS $pp65_{495-503}$ or the $HIVgag_{77-85}$ CD8+ T-cells as a percentage of all IVS CD8+ T-cells in healthy volunteers vaccinated with CMV peptide + PF-03512676



Median levels of $CD8^+$ T-cells specific for either the $pp65_{495-503}$ (filled symbols) or the $HIVgag_{77-85}$ (control, open symbols) tetramers are shown as a function of CMV peptide+ PF-03512676 vaccinations in healthy volunteers. p value was calculated by Wilcoxon rank-sum test to assess the significance of post-vaccination binding difference between $pp65_{495-503}$ and control ($HIVgag_{77-85}$) tetramer in the IVS expanded $CD8^+$ T cells.

In healthy volunteers, a dose response to the vaccine CMV peptide component was not apparent. However data collected from healthy volunteers immunized with Tet-CMV + PF-03512676 strongly support further evaluation of this vaccine in the HCT setting, due to its satisfactory safety profiles (Table 2.4.1a) and favorable immunogenicity (Figure 2.4.1b, right plot)[8].

2.4.2 Phase Ib pilot trial (COH IRB 12022) of CMVPepVax in HCT recipients

The completed Phase Ib trial in HCT recipients (COH IRB 12022; NCT01588015@www.clinicaltrials.gov) was performed to evaluate safety and immunogenicity of two injections of CMVPepVax (2.5 mg Tet-CMV + 1.08 mg PF-03512676), in HLA A*0201 CMV-positive adult recipients of an HLA-matched allogeneic HCT for hematologic malignancies. The primary study objective is safety, with secondary objectives including immunologic responses of HCT recipients to CMVPepVax.

Participants in this open label trial are randomized to two arms, the CMVPepVax vaccine arm and the observational arm. HCT recipients in the vaccine arm are injected twice on days 28 and 56 post-HCT with CMVPepVax, during the critical period in which primary CMV reactivation most predictably occurs (~day 40-100)[35, 67]. As of January 15th, 2015, CMVPepVax has been safely administered to 18 HLA A*0201 R^+ participants (total 32 vaccine injections). In order to meet the goal of randomizing 36 participants on Day 28 post-HCT, 46 participants were enrolled pre-HCT, ten of whom did not meet the Day 28 post-HCT vaccine administration criteria.

Eligible CMV positive HCT recipients (R^+) were HLA-A*0201, between 18-75 years (y) receiving allogeneic T-replete HCT for hematologic malignancy from matched-related (MRD) or 8/8 and 7/8 matched unrelated donors (MUD), excluding those with acute leukemia not in remission. The median age was 50

years (range: 19-75), with 24 (52%) donor CMV positive, 22 (48%) donor CMV negative, and 22 (48%) MRD to 24 (52%) MUD.

Safety of CMVPepVax in HCT recipients: Adverse events

Interim results from 17 (out of 18 planned) vaccinated R^+ indicate excellent tolerability and safety of CMVPepVax in all vaccinated HCT patients, and no patient met the safety stopping rule per-protocol. As shown in Table 2.4.2, there were no serious SAE or any AE exceeding Grade 2 that are definitely, probably, or possibly related to the vaccine formulation in the CMVPepVax treatment arm.

Table 2.4.2 List of SAEs in CMVPepVax vaccinated HCT recipients (N=18-)

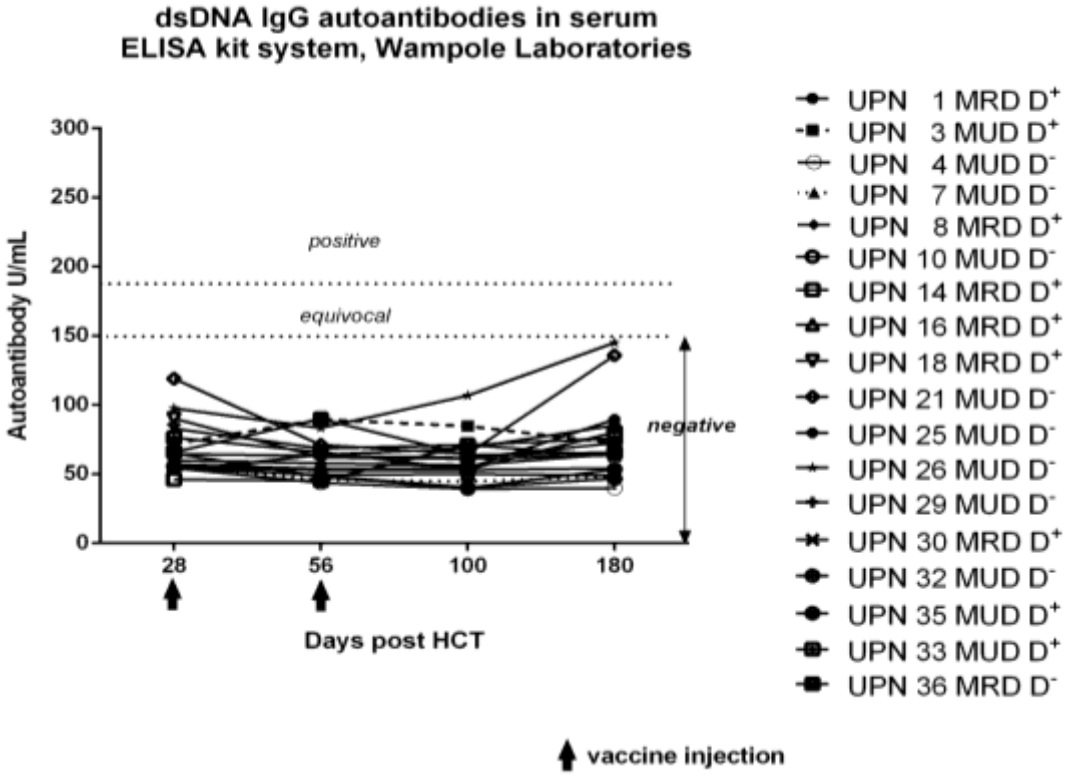
Patient Identifier	Date of Event	Outcome	Resolved	Adverse Event Term	Attribution
UPN 08	05/21/2013 (day of vaccination)	Hospitalization – Initial	05/22/2013	Grade 1 Fever	**Probably related**
UPN 07	05/31/2013 (30 days post vacc.)	Hospitalization – Initial	06/06/2013	Grade 3 Device Related Infection	Unlikely related
UPN 26	04/28/2014 (7 days post vacc.)	Hospitalization – Initial	05/01/2014	Grade 3 Diarrhea	Unlikely related
UPN 29	05/29/2014 (30 days post vacc.)	Hospitalization – Initial	06/06/2014	Grade 3 Nausea	Unlikely related
UPN 34	08/07/2014 (7 days post vacc.)	Hospitalization -Initial	08/20/2014	Grade 2 Nausea	Unrelated

Safety of CMVPepVax in HCT recipients: double stranded DNA autoantibodies

Sera from vaccinated HCT recipients were longitudinally evaluated during the 6 month observation period for levels of double stranded (ds) DNA autoantibodies. These analyses were performed since mouse models of systemic autoimmune diseases have indicated involvement of TLRs in the generation of autoreactive immune responses[85]. The semi-quantitative detection of human dsDNA IgG autoantibodies in serum was performed using the dsDNA ELISA kit system, by Wampole Laboratories (Princeton, NJ) at COH. Interestingly, none of the 14 R^+ patients vaccinated with CMVPepVax showed levels of dsDNA autoantibodies above the positivity threshold (>180 AAU/ml). Furthermore, none of them reached the so called equivocal threshold (>150 AAU/ml, Figure 2.4.2a) being all levels <150 AAU/ml.

These results in HCT recipients are distinct from the results from healthy volunteers in which transient increases in levels of dsDNA autoantibodies were detected in CMVPepVax vaccinated healthy adults, This difference is likely due to defects in cellular and humoral immunity which persist for over one year after HCT, with delayed immune reconstitution especially marked for B cells[86, 87].

Figure 2.4.2a Longitudinal profiles of dsDNA IgG autoantibody levels (AAU/ml) in sera of HCT recipients vaccinated with CMVPepVax

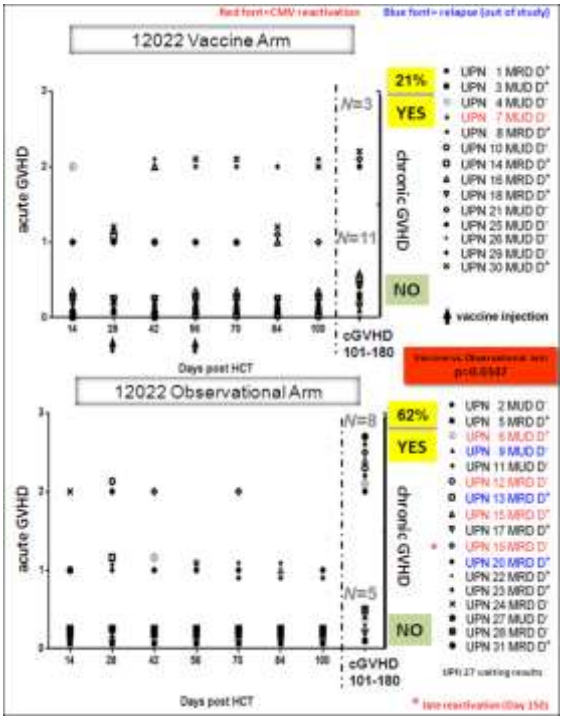


Longitudinal profiles of sDNA autoantibody levels (AAU/ml) in sera of HCT patients (UPN) vaccinated with CMVPepVax. Segmented lines indicate the ELISA thresholds. ELISA kit system, Wampole Laboratories.

Safety of CMVPepVax in HCT recipients: Impact on GVHD

Importantly, there was no increased incidence of aGVHD, in patients immunized with CMVPepVax. An unexpected and striking finding was the difference in rates of cGVHD in patients who reached day 180 in the vaccine arm (3/11) vs. the observational arm (8/13, p=0.0347), as shown in Figure 2.4.2b. Noteworthy is that the vaccine formulation is more likely to stimulate TH1 responses, yet TH2 immunity is mechanistically associated with cGVHD. Additionally, CpG DNA PF03512676 the adjuvant in CMVPepVax is known to leading to potent immunostimulatory effects, such NK cell activation [63] which have been described to be inversely correlated to cGVHD onset [88]. Thus CMVPepVax vaccinated HCT recipients could have higher levels of NK cells limiting cGVHD.

Figure 2.4.2b aGVHD and cGVHD in HCT recipients vaccinated with CMVPepVax and control HCT patients



aGVHD and cVHD in vaccine (upper plot) and observational (lower plot) HCT patients (UPN). t test was used to assess the difference (p value in red box) between groups.

Safety of CMVPepVax in HCT recipients: Impact on Disease Relapse

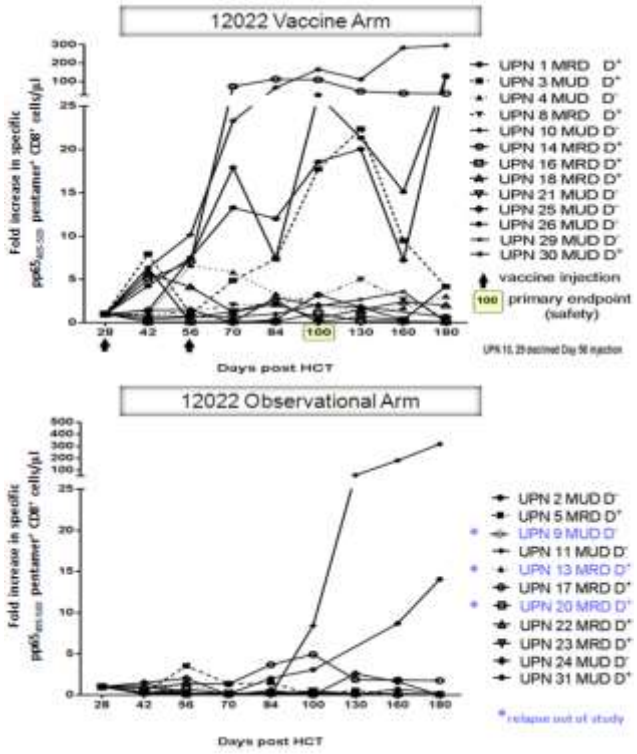
Interestingly, all CMVPepVax vaccinated recipients continue to remain cancer free, while 3 observational patients experienced relapse of their hematological malignancy. Recent observations relating CMV reactivation and reduced relapse have driven strong interest in a mechanistic understanding of the phenomena observed in this trial related to CMV vaccine or the adjuvant, PF03512676 [18-20, 89].

CMVPepVax in HCT recipients: Immunogenicity studies

Vaccine specific immune responses have been evaluated by monitoring CMV-specific $CD8^+$ T cells, using multi-color flow cytometric analysis. Levels of $CD8^+$ T cells (cells/µL) binding to CMV-$pp65_{495-503}$ HLA *0201 pentamers (Proimmune, Oxford. UK) have been measured for thirty-one participants; fourteen of them were from the CMVPepVax vaccine intervention and seventeen from the observational arm. All HCT recipient participants vaccinated with CMVPepVax showed a measurable and consistent increase in pp65 levels post-vaccination, as plotted in Figure 2.4.2c (upper plot).

This pattern is consistent with a vaccine effect, and a formal statistical analysis has been performed. In nine vaccine arm patients and in ten observational patients who reached day 180 without CMV reactivation, increases in CMV pp65 levels after first and second injections were compared using generalized estimating equations. The model indicates an average 6.5 fold positive vaccine effect (p=0.02). Thus CMVPepVax significantly expanded CMV-pp65 CD8 T cells which have been described to protect HCT recipients from CMV complications [9, 10].

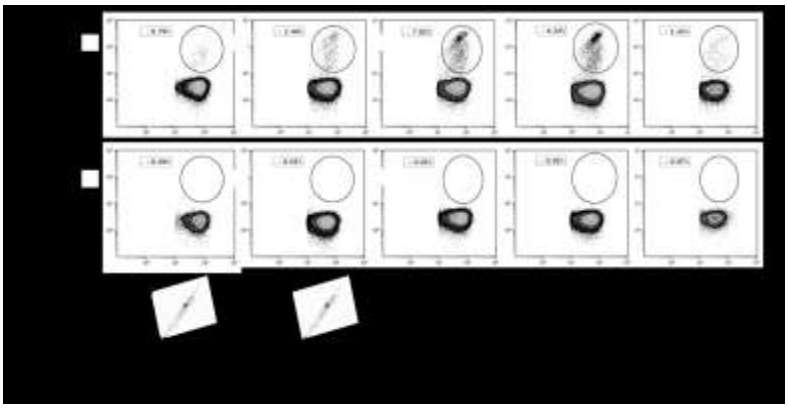
Figure 2.4.2c CMV-pp65 $_{495\text{-}503}$ pentamer CD8+ T cell amplification in HCT recipients vaccinated with CMVPepVax



Increase in specific CMV-pp65 $_{495\text{-}503}$ T cells, during the first 6 months post-HCT. Vaccine arm UPN 07, and observational arm UPN 06, 12, 15, 19, 27 and 28 who CMV reactivated are not included in this figure.

Moreover, CMV-pp65$_{495\text{-}503}$ T cells levels remain sustained through day 180 as shown in the representative FACS plots of HLA A*0201 CMV pp65 and HIV gag (control) pentamer binding assay, in CMVPepVax vaccinated UPN 8 (Figure 2.4.2d).

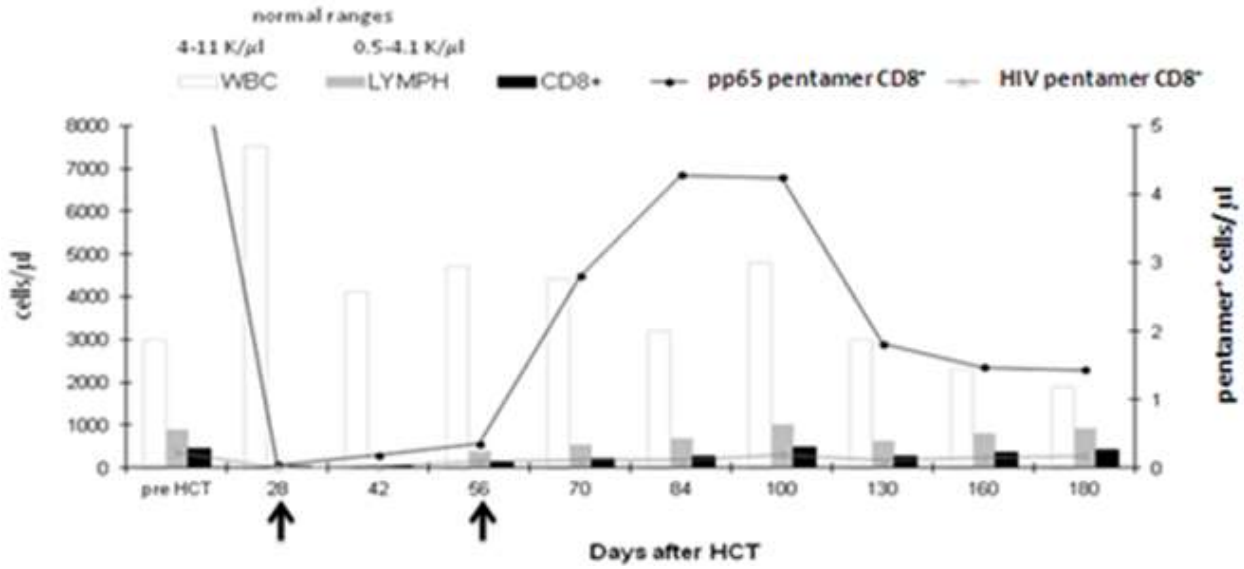
Figure 2.4.2d CD8 T cells binding to CMV pp65 and HIV gag (control) MHC class I pentamers in a representative HCT recipient vaccinated with CMVPepVax (UPN8)



FACS plots of CD8 T cells specific for CMV pp65 and HIV gag (control) MHC class I pentamers (Proimmune®, Oxford, UK) in CMVPepVax vaccinee UPN8. CMV pp65 CD8 T cells are detected up to 180 days after HCT.

The steady increase in levels of CMV-pp65 $_{495\text{-}503}$ T cells consistently follows vaccine injection time points, and appears to diminish at later time points post-vaccination, as seen in representative HCT recipient vaccinated with CMVPepVax UPN 14 (Figure 2.4.2d).

Figure 2.4.2d Kinetics of CD8+ T cells/ul binding to CMV- pp65 and HIV gag (control) MHC class I pentamers in a representative HCT recipient vaccinated with CMVPepVax (UPN 14)



CD8 T cells/ul specific for CMV pp65 and HIV gag (control) MHC class I pentamers (Proimmune®, Oxford, UK) in CMVPepVax vaccinee UPN8. CMV pp65 CD8 T cells are detected up to 180 days after HCT. UPN 14 is D+MRD, A 0201/0202, B 5204/5301.

CMVPepVax in HCT recipients: CMV reactivation

Though the current safety and immunogenicity trial was not powered to assess differences in CMV reactivation rates, we did observe a 5.5 fold CMV reactivation decrease in CMVPepVax vaccinated HCT recipients (1/16=6%) compared to the observational arm patients, in whom expected CMV reactivation rates of 33% (6/18) were reached[67, 90].

In conclusion, trial IRB 12022 results indicate safety of injecting CMVPepVax in HCT recipients on day 28 and day 56, with no increase in aGVHD, and reduced CMV reactivation associated with vaccine-stimulated immunity. Compelling observations of reduced cGVHD and relapse requires further testing in the proposed Phase II efficacy trial, which has been NIH-funded as a multi-institution clinical trial.

Immune reconstitution in HCT recipients vaccinated with CMVPepVax

We assessed the phenotypes and time-course of the pp65-specific CD8 T cell subsets that expanded in response to CMVPepVax vaccination. The functionality and antiviral role of CMV-specific T cells have been linked to immune reconstitution profiles characterized predominantly by differentiated effector memory T (TEM) subsets that have lost membrane expression of the costimulatory molecule CD28, and often re-express the RA isoform of CD45 (TEMRA). Major histocompatibility complex class I pp$_{65495–503}$ multimers, as well as CD28 and CD45 memory markers, were used to detect immune reconstitution in blood specimens from HCT recipients enrolled in the Phase 1b clinical trial. Specimens from the 10 (out

of N=18) vaccine patients who had adequate (≥0.2%) multimer binding to allow for memory analysis showed highly differentiated TEM and TEMRA phenotypes for $pp_{65495-503}$-specific CD8 T cells during the first 100 days post-transplant. In particular, by day 70, during the period of highest risk for CMV reactivation, combined TEM and TEMRA phenotypes constituted a median of 90% of $pp_{65495-503}$-specific CD8 T cells in these vaccinated patients. CMV viremia was not detectable in the CMVPepVax patients, although their $pp_{65495-503}$-specific CD8 T cell profiles were strikingly similar to those observed in viremic patients, who did not receive the vaccine. Collectively, our analysis indicates that, in the absence of clinically relevant viremia, CMVPepVax reconstituted significant levels of differentiated effector memory $pp_{65495-503}$-specific CD8 T cells early post-HCT. The body of data from this current study indicates that the rapid reconstitution of CMV-specific T cells, with marked levels of effector phenotypes may have been key to the favorable outcomes of the CMVPepVax clinical trial. ([91], [92])

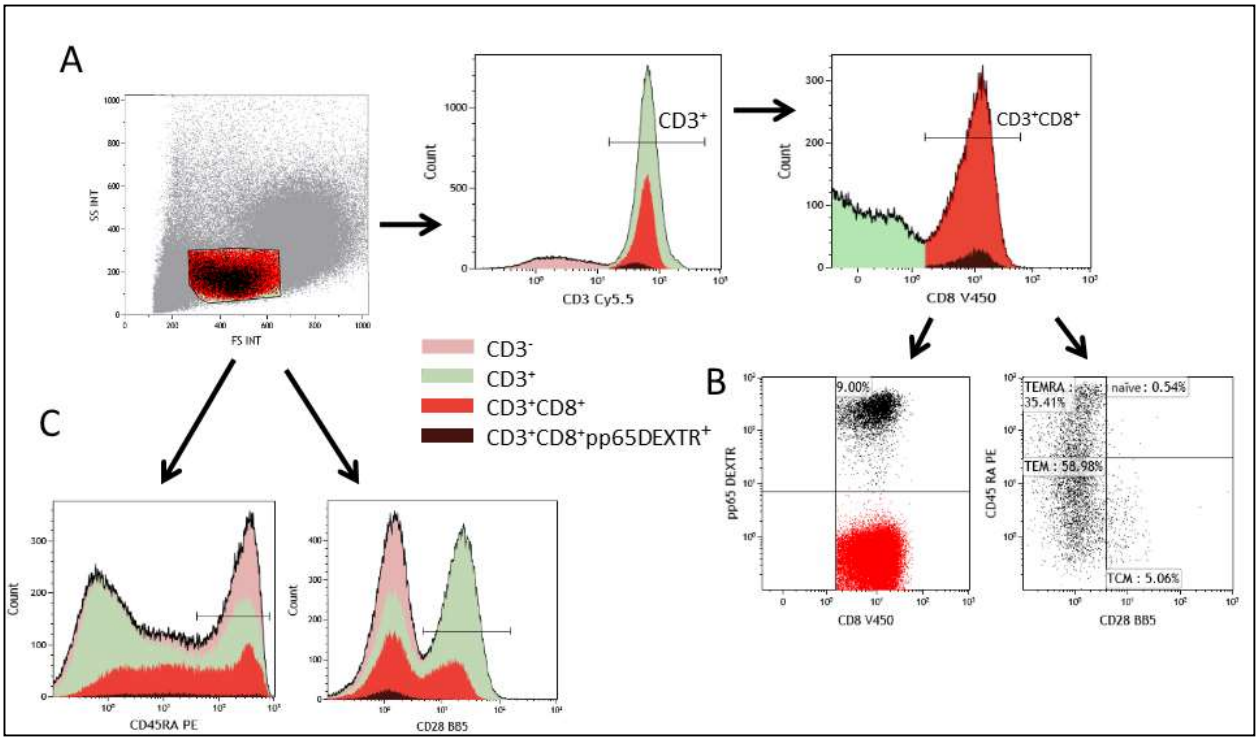


Figure 1. Gating strategy for identifying the memory phenotype of pp65-specific T cells. Representative data plots for VA non-viremic UPN 8 at day 70 post-HCT, analyzed with Kaluza software 1.5, are shown. A primary lymphocyte gate was set on forward and side scatter (FS INT vs. SS INT) and subsequent gates set on $CD3^+$ and $CD8^+$ populations (**Panel A**). The left plot of **Panel B** shows the quantification of $pp65_{495–503}$-specific $CD3^+$ $CD8^+$ T cells based on binding to APC-conjugated dextramers incorporating the $pp65_{495–503}$ peptide (pp65 DEXT). The right plot of **Panel B** shows the T cell memory populations defined according to CD28 and CD45RA expression as follows: naïve T cells ($CD28^+$ $CD45RA^+$, upper right quadrant); central memory (TCM, $CD28^+$ $CD45RA^-$, lower right quadrant); effector T cells (TEM, $CD28^-$ $CD45RA^-$, lower left quadrant); and effector T cells (TEMRA, $CD28^-$ $CD45RA^+$, upper left quadrant). **Panel C** histogram plots show the of CD45RA and CD28 expression of the gated lymphocyte populations. The T cell populations are color coded as follows: $CD3^-$ in pink, $CD3^+$ in green, $CD3^+$ $CD8^+$ in red and $CD3^+$ $CD8^+$ $pp65_{495–503}$ $dextramer^+$ in black.

2.5 Interim analysis and rationale for continuation of the trial

The interim analysis for the primary endpoint (Section 12.4 Data Analysis Plan) unexpectedly showed that more CMV events observed on the vaccine arm (5 events) than on the placebo arm (3 events), thus

meeting the futility stopping rule. Accordingly, the study held further accrual and continued to treat/monitor patients (total N= 60) who have been consented and enrolled prior to the interim analysis data became available. The study statistic team derived the following data on the interim analysis based on 48 patients.

Table 2.5 Interim analysis of IRB#13494

N=48 pts with immunologic data	CMVPepVax (n=24)	Placebo (n=24)
CMV PCR >500 or Disease through d100 (Primary)	**5***	**3**
Median days of CMV reactivation *(in patients who had reactivation)*	**17**	**42.5**
Median days of anti-viral therapy through d100 *(in patients who had reactivation)*	**37.5**	**36**
Median days of anti-viral therapy through d365 *(in patients who had reactivation)*	**46**	**38**
Relapse through D365 (or last f/u)	**4**	**5**
Death through D365 (or last f/u)	**4**	**3**
Acute GVHD grade 2-4	**10**	**10**
Acute GVHD grade 3-4	**0**	**5**
# of patients who achieved 4-fold increase in pp65-dex from d28 to d56	**5**	**1**
# of patients who achieved 2-fold increase in pp65-dex from d28 to d56	**8**	**4**
# of patients who achieved 4-fold increase in pp65-dex from d28 to d84	**9**	**5**
Mean relative change from d28 to d56	**mean=.70; sd=1.6**	**mean=.10; sd=.93**
Mean relative change from d28 to d84	**mean=.75; sd=1.5 n=23**	**mean=.56; sd=1.2 n=22**

(*) Includes a patient who met reactivation criterion but resolved w/o antivirals.

(**) Events through d365 include events before d100.

(***) last day – first day

Unanticipated features of the interim analysis:

- The overall CMV reactivation rate was lower than past clinical experience at COH, yielding only 8 events at the interim analysis. If the vaccine was effective, 13 events would be expected. If it were ineffective, 19 events would be expected. The lower overall event rate increases the chance of inappropriately stopping at the interim analysis from about 2% as originally planned, to about 27%, assuming the vaccine still prevents half of all CMV reactivations.

- A second unanticipated feature was the early timing of most reactivations: 7 of the 8 events occurred before day 100 which is prior to the second vaccination (scheduled for day 56).

Safety and immunologic data at the interim analysis:

- There have been no safety concerns to date. The immunologic recovery based on the number of patients achieving 2-fold or 4-fold increase in CMV-specific T cells was favorable in the vaccine arm (Table 2.5).

Rationale/justification for continued accrual:

1) For the entire cohort of 97 patients

After the interim analysis the trial was redirected toward the combination approach with the currently approved Prevymis prophylaxis regimen as a result of still unmet need to address the rise in CMV reactivation past week 18 despite the use of the antiviral.

The combined reactivation rate at this point was 8/48, or 17%, very close to the 15% rate that was hoped for on the vaccine arm, but much lower than expected for the placebo arm. At such low reactivation rates, the futility rule has a much higher false alarm rate than planned. If the vaccine offers the same degree of protection originally planned, (i.e. preventing 25 of 40 reactivations) then the futility rule would have a false alarm rate of approximately 27%. The interim result is thus not inconsistent with the original targeted effect size, and does not provide a satisfactory contradictory signal to the success seen in protocol IRB#12022.

There will be a useful gain in clinical and scientific knowledge by continuing the trial through the planned 97 patients even when the primary hypothesis test was futile at the low reactivation rates for the initial cohort.

In addition, there are further factors that likely contributed to the current results such as more liberal eligibility criteria in 13494 compared with the previous successful pilot trial (IRB#12022, detailed in section 2.4.2 above), and heterogeneity in CMV detection methods and preemptive therapy across participating sites, with some events occurring early. Therefore, to be more consistent with the original pilot trial IRB#12022, we will exclude <u>any</u> grade 3-4 aGVHD before randomization (day 28) and <u>any</u> level of detectable viremia prior to day 28. In addition, we will remove from efficacy analysis if a patient was found to be viremic on the day of vaccination (retroactive since the result will be available only after 1-2 days from the vaccination).

2) For the new cohort of 36 patients with the combination of CMVPepvax and Prevymis

The new cohort size is based on the original study plan budgetary constraints. The amended study design will allow for establishing a possible positive effect of CMVPepVax in patients who experience CMV reactivation during or after the administration of Prevymis – an antiviral agent used for CMV disease prophylaxis. Prevymis has been approved by the FDA for all CMV seropositive HCT recipients, and accordingly, the City of Hope HCT SOP has adopted the use of Prevymis.

While the interim results indicate that CMVPepVax is likely not a suitable alternative to Prevymis prophylaxis, there remains a need to prevent the significant risk of reactivation after anti-viral therapy ends on day 100. In addition, the period of prophylaxis represents an opportunity to achieve protective immunity ahead of the early reactivations that were observed in the absence of Prevymis prophylaxis. This need and opportunity motivate the amendment of the trial to address the effect of CMVPepVax administered during Prevymis prophylaxis.

There is no known or theoretical overlapping toxicity between CMVPepVax and Prevymis.

Clinical and scientific significance

- Late CMV reactivation after stopping Prevymis on day 100 remains a major clinical problem, which can potentially be overcome by the combination approach of CMVPepVax plus Prevymis.

Novelty

- The combination of CMVPepVax (developed at COH, IND held by COH) and Prevymis is a highly novel combination.

- CMV-specific T cell immune reconstitution has not been described in patients receiving Prevymis. Thus the immunologic data of patients receiving Prevymis, even in a small cohort of 18, are highly informative and novel.

- The clinical and immunological impact of CMVPepVax when combined with Prevymis has not been studied. The proposed amendment allows better understanding the vaccine-induced immunologic protection for CMV reactivation occurring after day 100.

3.0 PARTICIPANT ELIGIBILITY CRITERIA

3.1 Pre-HCT Inclusion Criteria

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

Informed Consent and Willingness to Participate

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

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

Age Criteria

___ 3. Age 18 to 75 years.

Nature of Illness and Transplant Related Criteria

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

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

___ 5. HLA A*0201 High resolution, 4-digit typing is required at HLA-A2 to ensure A*0201 status.

___ 6. CMV seropositive (recipient)

___ 7. Planned related or unrelated HCT, with HLA donor allele matching. Related donor must be an 8/8 match for HLA-A, -B, and –C at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing. Unrelated donor must be an 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Patients undergoing a second allo HCT are not eligible (patients who have undergone a previous autologous HCT are eligible).

___ 8. Planned HCT with no ex-vivo T cell depletion of graft. Conditioning and immunosuppressive regimens according to institutional guidelines are permitted.

Clinical laboratory parameters

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

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

Child Bearing Potential

___ 11. Agreement by females of childbearing potential **and** sexually active males to use an effective method of contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for up to 90 days post-HCT. PF-03512676, the adjuvant co-injected with the CMV peptide, has been determined to be embryolethal and teratogenic in animal testing. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

3.2 Pre-HCT Exclusion Criteria

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

Previous therapies

___ 1. Any prior investigational CMV vaccine

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

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

___ 3. Live attenuated vaccines

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

___ 5. Allergy treatment with antigens injections

___ 6. Alemtuzumab or any equivalent in vivo T-cell depleting agent. This includes ATG and post-transplant cyclophosphamide.

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

___ 8. Other investigational product – concurrent enrollment in other clinical trials using an investigational product is prohibited

___ 9. Other medications that might interfere with the evaluation of the investigational product

Other illnesses or conditions

___ 10. Patients with active autoimmune conditions requiring systemic immunosuppressive therapy within the previous 5 years are not eligible

___ 11. Pregnant women and women who are lactating. CMVPepVax has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the administered vaccine, breastfeeding should be discontinued if the mother is enrolled on this study.

___ 12. 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

___ 13. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

3.3 Participation of Special Populations

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

4.0 PARTICIPANT ENROLLMENT AND RANDOMIZATION

4.1 Pre-Enrollment Informed Consent and Screening Procedures

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

4.2 Participant registration

4.2.1 COH DCC Availability and Contact Information

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

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

4.2.2 Slot verification and reservation

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

4.2.3 Registration procedure

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

1. The participating site's data manager/coordinator/research nurse should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window (60 days to 0 days before planned HCT).
2. The data manager/coordinator/research nurse should then e-mail copies to DCC@coh.org of the following documents to the DCC:

- Registration Cover Sheet (Appendix H)
- Completed Eligibility Criteria List (printed from Section 3.1-2 of the protocol)
- Source documentation to support eligibility criteria**
- Signed informed consent document
- Signed HIPAA authorization form (if separate from the informed consent document)
- Signed subject's Bill of Rights (COH only)

**For COH participants, provide copies of source documentation only if not readily available as a finalized record in the COH EMR

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

4.3 Randomization

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

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

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

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

Only participants who receive a vaccination (CMVPepVax or Placebo) will be considered "randomized".

4.4 Emergency De-Blinding Procedures

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

The following procedure should be followed:

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

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

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

5.0 TREATMENT PROGRAM

5.1 Treatment Overview

The study intervention will consist of a vaccine administration of either CMVPepVax (N=48) or placebo (N=48), depending on participant randomization. All patients in the new cohort (36 patients, 18 vs 18) will receive Prevymis as a standard of care.

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

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

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

All participants in the new cohort (36 patients, 18 vs 18) will be assigned to Prevymis standard of care group and will receive Prevymis at the dose of 240 or 480 mg per day for approximately 14 weeks, up to day 100 post-HCT.

5.2 Maintaining blinded randomization

The vaccine treatment assignment will be masked from patients and health care providers, but known to the DCC coordinators, study monitors, auditors, pharmacy personnel, and the statistician. Due to differences in appearance of the active agent and the placebo, the person administering the vaccine (placebo or active agent) should limit discussions regarding the characteristics regarding the agent's appearance or administration (i.e. ease or difficulty of administration) to the study pharmacist. Since the person administering the vaccine may detect a difference in appearance between placebo or active agent, he/she will be furthermore excluded from performing any protocol-required procedures or providing day-to-day medical care of the participant for the duration of the trial. The pharmacist will keep the treatment assignments and accountability documentation such that the documents cannot be

accessed by the individuals who conduct protocol-required assessments, follow-up assessments, or those involved in the day-to-day medical care of the subjects during the trial. Jennifer Drake will be the designated un-blinded PMT contact for Pharmacy. Disclosure of any knowledge of the randomization status to persons other than those permitted to know the randomization status would result in a protocol violation.

5.3 Vaccine Administration and Pre-Vaccination Prophylaxis

Note: the vaccine must be administered within 90 minutes of start of vaccine preparation. As such, coordination between the pharmacy and the staff administering the vaccine is essential.

5.3.1 Pre-vaccination preemptive therapy

At the discretion of the treating investigator and/or participant, within 15 minutes prior to vaccine administration a single dose of oral acetaminophen (Tylenol) at a recommended dose of 500 mg may be administered to the participant.

While allergic reactions were seen in healthy volunteers receiving CMVPepVax, no allergic reactions were seen in HCT recipients receiving CMVPepVax even in the absence of diphenhydramine. Diphenhydramine (Benadryl) 25 mg oral, or IV if unable to give orally, may be administered within 15 minutes prior to vaccine administration at the discretion of the treating investigator.

5.3.2 Vaccine administration and post-administration monitoring

The time limit that may not be exceeded when the agent should be administered will be noted on the vial label, or accompanying pharmacy documents.

During the agent administration process, the syringe should remain unseen by the participant to help maintain the study blind status. The syringe containing a 1 ml final volume will be administered subcutaneously in the upper arm, per standard clinical practice. Following administration, the participant will be monitored for 30 minutes for apparent vital sign changes, and local and systemic reactogenicity.

The time when the agent was administered (as noted on the vaccine label or accompanying pharmacy documents) and the time of vaccination will be noted in the medical record.

5.4 Assessments

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

5.4.1 GVHD assessment and performance status

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

5.4.2 CMV monitoring

For CMV monitoring, standard qPCR clinical laboratory methods will be required to evaluate CMV viral load and possible agent failure at least once per calendar week (usually twice weekly) or as required by SOC from day 21 to 100. From day 100 to day 180, CMV viral load will be monitored at least once every 2 weeks.

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

Clinical CMV disease status will be documented at each study visit, which may include the absence or presence of suspected CMV disease. When clinically indicated and per SOC, CMV disease will be assessed and, when present, the site (upper GI, lower GI, other, specify) and method of detection in the tissue (tissue culture, pathology etc.) will be documented. Presentations or suspected presentations of CMV disease in the absence of qPCR ≥500 gc/ml will be evaluated by the treating investigator in conjunction with the blinded PMT before a determination is made.

5.4.3 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 ≥ 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.

5.4.4 Disease relapse

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

5.4.5 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.

5.4.6 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

5.4.7 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.

5.4.8 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, using COH and participating sites documentation guidelines. 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 dosage for the 7 days prior to vaccine administration
- Prohibited medications

5.4.9 Immunogenicity testing

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

5.5 Criteria for Completing/Discontinuing Study Participation

Participation may continue until one of the following criteria applies:

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

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

5.6 Follow-Up and Duration of Participation

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

5.6.1 Participants who do not receive Day 28 vaccine administration

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

5.6.2 Participants who reactivate CMV on Day 28

Participants who receive Day 28 vaccination, but have detectable levels of CMV in Day 28 test sample will not be included in the analysis.

5.6.3 Participants who relapse after receiving vaccine administration

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

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

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

– may elect to continue study monitoring procedures without Day 56 vaccine (if not already administered).

– may elect to continue to be monitored for survival (including reason for mortality) until Day 365 only, or
– may elect to withdraw completely; further follow-up or assessments will not occur.

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

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

5.7 Supportive Care, Other Concomitant Therapy, Prohibited Medications

Optional preemptive treatment prior to vaccine administration is detailed in Section 5.3.

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

- No other investigational agents may be given to patients
- Alemtuzumab or any equivalent in vivo T-cell depleting agent is not permitted in this study following HCT, because its administration results in *in vivo* depletion of B, T and dendritic cells, potentially negating any positive effect of vaccinating the recipient with CMVPepVax.
- Prophylactic therapy with CMV immunoglobulin or prophylactic antiviral CMV treatment (GCV/VAL, FOS, Cidofovir, CMX-001) is not allowed following HCT with the exception of Prevymis administration in Prevymis cohort. GCV/VAL, FOS, Cidofovir, CMX-001 may be used according to institutional SOC for preemptive management of CMV viraemia. In general, preemptive therapy should not commence until after CMV qPCR ≥500 gc/ml; for preemptive therapy for qPCR<500, the study PIs are to be consulted.
- Prophylatic 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 it will not be allowed in this study following HCT. Therapeutic use of these agents is permitted per institutional standard practice. Acyclovir has no therapeutic efficacy against CMV and is allowed as standard of care to prevent HSV.
- Medications that might interfere with the evaluation of the investigational product are prohibited up to 14 days after the second vaccination (day 70 post-HCT). Medications in this category include, but are not limited to:
    - Live attenuated vaccines
    - Medically indicated subunit (Engerix-B for HBV; Gardasil for HPV) or killed vaccines (e.g. influenza, pneumococcal, or allergy treatment with antigen injections)
- Steroid therapy with prednisone, or equivalent, is permitted (see section 6.0 for specific steroid dose criteria for vaccinations).

6.0 VACCINE ADMINISTRATION CRITERIA

6.1 Day 28-Post-HCT Vaccine Administration Criteria

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

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

___ 1. NOT experienced ≥ Grade 3 GVHD between days 0-28 post HCT

___ 2. NO detectable viremia between days 0-28 post HCT

___ 3. Disease has NOT relapsed since HCT

___ 4. Successful primary engraftment WITHOUT secondary graft failure

___ 5. NO ongoing post-HCT ≥ Grade 3 non-hem AE's, with the exception of grade 3 glucose intolerance, cholesterol, triglyceride, and hyperglycemia

___ 6. Negative for CMV viraemia within the past 7 days

___ 7. Negative for CMV end organ disease (biopsy proven) post-HCT

___ 8. All prednisone doses within the past 7 days have been ≤ 1 mg/kg/day (or prednisone equivalent)

___ 9. NOT received any prohibited medications (Section 5.7)

___ 10. Negative pregnancy test result for females of child bearing potential

___ 11. For Prevymis cohort patients: patients who received Prevymis for at least 7 days before day +28 post HCT

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

6.2 Day 56-Post-HCT Vaccine Administration Criteria

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

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

___ 1. NOT experienced Grade 4 GVHD since Day-28 vaccination

___ 2. NO Grade 3 GVHD within the past 7 days

___ 3. Disease has NOT relapsed since HCT

___ 4. NO secondary graft failure

___ 5. NO ongoing post-HCT ≥ Grade 3 non-hem AE's, with the exception of grade 3 glucose intolerance, cholesterol, triglyceride, and hyperglycemia

___ 6. All prednisone doses within the past 7 days have been ≤ 1.5 mg/kg/day (or prednisone equivalent)

___ 7. NOT received any prohibited medications (Section 5.7)

___ 8. Negative pregnancy test result for females of child bearing potential

7.0 ADVERSE EVENTS LIST AND REPORTING OF UNANTICIPATED PROBLEMS AND ADVERSE EVENTS

7.1 Toxicities to CMVPepVax

7.1.1 Expected (known) toxicities to CMVPepVax

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

Arthralgia (grade 3), Chills (grade 1), Fever (grade 2), Malaise (grade 3), Allergic reaction (grade 3), Myalgia (grade 1), Nausea (grade 2), Vomiting (grade 2), Headache (grade 2), Cutaneous reaction (pruritus, erythema) (grade 1)

7.1.2 Anticipated toxicities to CMVPepVax

Anticipated toxicities that have not yet been seen from the agent but are foreseeable based on other similar agents include:

Bruise at the site of injection, hypotension.

7.2 Toxicities to the Placebo

7.2.1 Expected (known) toxicities to placebo

There were no (known) toxicities associated with administering neither vaccine norplacebo.

7.2.2 Anticipated toxicities to placebo

Anticipated toxicities that have not yet been seen from the placebo but are foreseeable based on other similar agents include:

Bruise at the site of injection, hypotension.

7.3 Definitions

7.3.1 Adverse event (AE)

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

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

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

7.3.3 Expected Adverse Event

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

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

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).

7.3.5 Unanticipated problems Involving Risk to Subjects or Others

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

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

7.3.6 AE Description and Grade

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

7.3.7 AE Attribution

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

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

by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.

- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

7.4 Routine Reporting of Adverse Events by Site Investigators

Routine AE reporting will occur via data entry into the study eCRF. Adverse events experienced from the time of initial study treatment (Day 28 Post-HCT vaccine administration) to Day 100 post-HCT or until disease relapse (whichever occurs sooner) will be reported into the eCRF. AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.

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

7.5 Expedited Reporting of Unanticipated Problems and SAEs by Site Investigators

Each adverse event will be assessed to determine if it meets the criteria for reporting Adverse event reporting is to occur according to the site's specific IRB guidelines, and as outlined in this Section.

7.5.1 Adverse Event Reporting to Local IRB

Sites are to report to their local IRB per their site's specific institutional and IRB guidelines. As soon as possible, non-COH sites will provide to the Data Coordinating Center copies of the IRB submission and corresponding IRB response.

7.5.2 Adverse Event Reporting to Coordinating Center/ Study PI

Adverse events that meet the specified below are to be reported to the Data Coordinating Center and Study PI within the timelines and per the procedures in the sections that follow.

Rationale for reportable events is derived from FDA expedited reporting criteria, COH reporting criteria, and Pfizer reporting criteria.

Report the following to the Coordinating Center/Study PI within 24 hours of being aware that the event met reporting criteria:

Report the following from the signing of consent to study completion

- All unanticipated problems

Report the following from first vaccine administration up to 30 days after the last dose of vaccine

- All serious adverse events regardless of relationship to study agent, study procedure, underlying disease or concomitant treatment
- AEs that meet the definition of an unanticipated problem

Report after 30 days after the last dose of vaccine

- All serious adverse events that are considered possibly, probably, or definitely related to the study agent.

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

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

How to report an SAE/UP to the Coordinating Center/Overall PI – Non COH Sites:

1. Document/describe AE/UP f the UP/SAE Coversheet. The SAE Coversheet is found in Appendix G. A modifiable Microsoft Word document is also available from the Data Coordinating Center. An electronic signature on the document will be accepted.

2. Scan and email above documents to DCC@coh.org with the subject title as "CMV SAE".

All SAE reports received at this account are forwarded immediately to study Principal Investigator, and to Coordinating Center personnel. While not required, if available and applicable, please also include the local IRB submission for this event in the submission.

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

How to report AEs to the Coordinating Center/Overall PI –COH Investigative Sites

1. Email the following information to DCC@coh.org and rnakamura@coh.org.
    a. Participant ID, date the event met criteria for reporting, 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.
2. Complete the iRIS AE/UP reporting form per COH reporting timeline

7.6 Reporting of Adverse events by the Study PI

The Study PI (or designee) will:

- Report to COH IRB and DSMC all reportable adverse events that meet COH IRB and DSMC reporting criteria and occur at non-COH sites according to City of Hope's Institutional policy on reporting adverse events and indicate whether or not a protocol and/or consent form change is required.
- Report all expedited reportable adverse events to participating investigators as an IND Safety Report occurring within 30 calendar days of receipt of sponsor (lead site) notification, and indicate whether or not a protocol and/or consent form change is required. A cover letter will indicate the protocol title, the IND#, whether the FDA was informed, and, for non-COH sites, a statement that the report should be submitted to their local IRB for review as an IND safety report if applicable per local IRB policy.
- Report to the FDA, (via COH Office of IND Development and Regulatory Affairs (OIDRA)) regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

- Report to Pfizer, (via COH OIDRA) regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.
- Submit annually within 60 days (via COH OIDRA) of the anniversary date of when the IND went into effect, an annual report to the FDA which is to include a narrative summary and analysis of the information of all FDA reports within the reporting interval, a summary report adverse drug experiences, history of actions taken since the last report because of adverse drug experiences.
- Report every three months to the COH DSMC a Protocol Management Team report, to include aggregate analysis of safety information and accrual and participant status.
- Circulate to all participating sites for submission to their IRBs the above Protocol Management Team (DSMC) report and DSMC recommendation, in accordance with NIH guidance.
- Report to Pfizer summary safety information every three months.
- Forward to participating sites all reportable adverse events to participating investigators as an IND Safety Report occurring within 30 calendar days of receipt of lead site notification, and indicate whether or not a protocol and/or consent form change is required. A cover letter will indicate the protocol title, the IND#, whether the FDA was informed, and, for non-COH sites, a statement that the report should be submitted to their local IRB for review as an IND safety report if applicable per local IRB policy.
- Forward to participating sites all IND safety reports received from Pfizer for the CMVPepVax adjuvant, PF-03512676, that have not occurred directly on this protocol, indicating whether a consent form or protocol change is required within 30 days of notification to Study PI.
- Submit to participating sites all IND safety reports for CMVPepVax, that have not occurred directly on this protocol, indicating whether a consent form or protocol change is required within 30 days of notification to Study PI.

8.0 AGENT INFORMATION

8.1 CMVPepVax and Placebo Vaccine –information applicable to vaccine components

CMVPepVax will be prepared by the investigational pharmacy from the following components provided by the Sponsor and detailed further in the sections that follow:

- Tet-CMV peptide
- PF-03512676 adjuvant
- Normal saline
- Sodium acetate (10mM)

The Placebo Vaccine will be prepared by the investigational pharmacy from the following components provided by the Sponsor and detailed further in the sections that follow:

- Neut™ (0.4%) Sodium Bicarbonate Additive
- Sodium acetate (10mM).

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

8.1.1 Accountability,

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

In addition, documentation of the preparation of the vaccine (placebo or CMVPepVax) is required. See Sections 8.6 and 8.7 for details.

The sponsor should be promptly notified of any deviations to the procedures related to the study agent.

8.1.2 Handling

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

8.1.3 Ordering

The COH IDS pharmacy will order all agents from the respective supplier (COH Center for Biomedicine and Genetics; Biopharmaceutical Development Program at the Frederick National Laboratory for Cancer Research, Pfizer) in coordination with and as approved by Dr. Diamond (ddiamond@coh.org) and Dr. Nakamura (rnakamura@coh.org).

External site Pharmacies will order all agents from the COH IDS pharmacy.

8.1.4 Destruction and Return

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

8.2 Tet-CMV, the synthetic peptide contained in CMVPepVax

Additional information about Tet-CMV can be found in the City of Hope CMVPepVax IB.

8.2.1 Description

Tet-CMV peptide (=Tetanus-CMV; CODE NAME: NSC-721434; IND, BB-13124) which consists of the HLA A*0201-restricted CMV $pp65_{495-503}$ epitope covalently attached to the tetanus toxin $tt_{830-843}$ epitope (P2).

Tet-CMV peptide is a 29 amino acid and 3052.6 dalton peptide. Its Molecular Formula is: Tet-CMV = $C_{137}H_{227}N_{35}O_{41}S_1$.Its Amino Acid Sequence is: Tet-CMV: H-Lys-Ser-Ser-Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Ala-Ala-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-OH. The Pharmacologic Class is: Peptide Vaccine.

8.2.2 Toxicology

Most common AEs included mild to moderate cutaneous reactions at the injection site. The duration of related Grade 1 and 2 AEs ranged from 1-2 days. No grade 3 AEs were reported with CMVPepVax, the same formulation selected to be used in HCT recipients (approx 2.5 mg Tet-CMV peptide co-injected with 1.08 mg of PF-03512676).

8.2.3 Supplier

Tet-CMV study drug is being provided to COH by the National Cancer Institute sponsored NExT (NCI-NExT) program, which contracted with Bachem Incorporated (Torrance, CA) for cGMP-grade production of Tet-CMV. COH Center for Biomedicine and Genetics (CBG) stores and supplies Tet-CMV to COH IDS pharmacy for this study. The CBG or COH IDS pharmacy will supply Tet-CMV to all external trial sites.

8.2.4 Form

The trial will initiate using the initial lot of Tet-CMV **(Lot#L0402009)**, which was used in IRB#12022. This was provided to COH by the National Cancer Institute sponsored NEXT (NCI-NEXT) program, which contracted with Bachem Incorporated (Torrance, CA) for cGMP-grade production of Tet-CMV. Tet-CMV is supplied frozen in 10 mM sodium acetate (pH 4.2), as a sterile, preservative-free, solution for SC injection at 11 mg/1.1 ml vial , and packaged in 2-ml USP Type I clear glass vials with gray butyl, FluroTec®-coated stoppers and aluminum flip-off seals. The new Lot of Tet-CMV will be supplied frozen at 3.3 mg/ml in 10 mM sodium acetate (pH 4.2), as a sterile, preservative-free, solution (fill volume 1.2 mL) packaged in 2-ml USP Type I clear glass vials with gray butyl, FluroTec®-coated stoppers and aluminum flip-off seals.

CMVPepVax administration provides a dose of 2.48mg Tet-CMV Peptide when the vaccine is prepared using the currently available Tet-CMV (LOT# L0402009, 11mg/1.1mL vial).

CMVPepVax administration provides a dose of 2.64 mg Tet-CMV Peptide when the vaccine is prepared using the new lot of Tet-CMV (LOT#L1501005, 3.3 mg/mL, fill volume 1.2 mL)

8.2.5 Storage and stability

Tet-CMV peptide is to be stored in a monitored freezer at -60°C to -90°C. Stability analyses of Tet-CMV are performed every 6 months.

8.2.6 Handling, Ordering, Accountability, Destruction and Return

See Sections 8.1

8.3 PF-03512676, the adjuvant of CMVPepVax

Additional information about PF-03512676 can be found in the Pfizer PF0351276 IB.

8.3.1 Description

Pfizer PF-03512676 (previously referred as CPG 7909 and CPG ODN 2006[66]) adjuvant is a pure substance named Agatolimod Sodium, and is classified as an investigational agent. PF-03512676 is an immunostimulatory single-stranded, phosphorothioate oligodeoxynucleotide containing four unmethylated deoxycytosine-deoxyguanine dinucleotide (CpG) motifs and synthesized with a nuclease-resistant phosphorothioate backbone. PF-03512676 is 24 nucleotides in length. Its structural formula is:

(3'-5')d(*P*-thio)(T-C-G-T-C-G-T-T-T-T-G-T-C-G-T-T-T-T-G-T-C-G-T-T) tricosasodium salt. Its molecular formula is: $C_{236}/H_{303}/N_{70}/O_{133}/P_{23}/S_{23}/Na_{23.}$

8.3.2 Toxicology

Use of PF-03512676 as a vaccine adjuvant at doses of 1.08 mg SC/injection results in an AE profile consisting of frequently reported mild to moderate injection site reactions and flu-like symptoms.

At dosing significantly higher than the dose/injection proposed in this trial, e.g., in monkeys the no-effect dose level for complement activation is 2-5 mg/kg. The toxicity of PF-03512676 includes effects of immune stimulation (liver and spleen enlargement and inflammatory infiltrates in rodents), activation of the alternative complement pathway and inhibition of the intrinsic coagulation pathway (shock-like syndrome in monkeys following rapid infusion), and dose-related histopathologic changes in liver and kidney during subacute and chronic administration. PF-03512676 had been determined to be embryolethal in rabbits and teratogenic in developing rats and rabbits.

8.3.3 Form

PF-03512676 is supplied as a sterile, preservative-free, isotonic, phosphate buffered saline solution at a concentration of 15 mg/ml, pH 7.4 and packaged in 2-ml USP Type I clear glass vials with gray butyl, FluroTec®-coated stoppers and aluminum flip-off seals. Each vial contains a total of 23.25 mg in 1.55ml, with 1.2 ml of extractable volume (18 mg/1.2 ml of PF-03512676).

CMVPepVax administration provides a dose of 1.06mg PF-03512676 when the vaccine is prepared using the currently available lot of Tet-CMV (LOT# L0402009, 11mg/1.1mL vial)

CMVPepVax administration provides a dose of 1.08mg PF-03512676 when the vaccine is prepared using the new lot of Tet-CMV (LOT#L1501005, 3.3 mg/ml, 1.2 mL fill volume)

8.3.1 Supplier

PF-03512676 is supplied as an investigational agent by Pfizer.

8.3.2 Storage and Stability

PF-03512676 will be stored under refrigeration (2 to 8°C) in a secure temperature controlled refrigerator. Stability tests on PF-03512676 are routinely performed by Pfizer.

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

See section 8.1.

8.4 Sodium acetate, the synthetic peptide diluent contained in CMVPepVax

Additional information about 10mM Sodium Acetate can be found in the City of Hope CMVPepVax IB.

8.4.1 Description

10 mM sodium acetate (CH_3COONa), pH 4.2 (Lot#L0910005) will be used as a diluent for CMVPepVax.

8.4.2 Toxicology

There are no data available on the toxicology of 10 mM sodium acetate, pH 4.2, administered by SC route. Sterile, nonpyrogenic, 40 mEq (2 mEq/ml) USP solution of sodium acetate are typically administered, after dilution, by the IV route as an electrolyte replenisher. The solution is intended as an alternative to sodium chloride to provide sodium ion (Na+) for addition to large volume infusion fluids for IV use.

8.4.3 Formulation

Sodium acetate will be supplied as a sterile, preservative-free, solution at 10mM, 1.1 ml volume, pH 4.2, and packaged in 2-ml USP Type I clear glass vials with gray butyl, FluroTec®-coated stoppers and aluminum flip-off seals.

8.4.1 Supplier

cGMP-grade sodium acetate solution (SAIC (NSC 733084)) will be supplied by Biopharmaceutical Development Program, NCI-Frederick in the formulation described above.

8.4.2 Storage and Stability

The sodium acetate 10mM vials will be stored under refrigeration (2 to 8°C) in a secure temperature controlled refrigerator. The sodium acetate 10mM solution has passed a safety test, sterility and stability tests, which are performed every 6 months.

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

See section 8.1.

8.5 Neut™ Sodium Bicarbonate Additive Solution for the Placebo Vaccine

Additional information about Neut™ Sodium Bicarbonate Additive Solution can be found in the City of Hope CMVPepVax IB and at

http://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=ca2906bc-7d85-449b-bc86-ed627bf69a9f

8.5.1 Description

Neut™ (0.4% sodium bicarbonate additive solution) is a sterile, nonpyrogenic solution of sodium bicarbonate in water which will be used to bring the pH of the placebo injection to a pH similar to CMVPepVax. Since the sodium acetate solution is at pH 4.2, addition of Neut™ will raise the pH of the placebo injection closer to a physiological pH.

Neut (0.4% sodium bicarbonate additive solution) is indicated for use as an additive to raise the pH of acid solutions administered intravenously to reduce the incidence of chemical phlebitis and patient discomfort due to vein irritation at or near the site of infusion.

8.5.2 Toxicology

There are no known warnings. It is administered by the intravenous route only after addition as a neutralizing agent to an acidic large volume parenteral solution. It is not for use as a systemic alkalizer.

8.5.3 Formulation

Neut™ will be supplied as Neut™ 0.4%, 1 ml volume packaged in a 2 ml flip-top vial. Neut™ 0.4% is formulated from Neut™ 4% diluted 1:10 in sterile water.

Each 5 mL of Neut™ 4% contains sodium bicarbonate 0.2 g (2.4 mEq each of Na+ and $HCO3^{-}$); edetate disodium, anhydrous 10 mg added as a stabilizer. Total sodium (Na+) content of each 5 mL is 56.1 mg

(11.2 mg/mL). The solutions contain no bacteriostat, antimicrobial agent or added buffer; pH 8.0 (7.0 to 9.5). Sodium Bicarbonate, USP is chemically designated as NaHCO3, a white crystalline powder soluble in water.

8.5.4 Supplier

Neut™ 4% sodium bicarbonate additive solution is produced by Hospira, Lake Forest, IL. Neut™4% sodium is supplied in 5 ml fliptop vials. The COH Center for Biomedicine and Genetics (CBG) formulates and supplies a 1:10 dilution of Neut™ in sterile water, which is vialed as Neut™ 0.4% (2mL fliptop vials). The COH CBG is a California Food and Drug Brand (CFDB) licensed manufacturing facility which operates under the principles of Current Good Manufacturing Practice regulations for the manufacture of phase I/II biologics. The COH IDS pharmacy will supply Tet-CMV to all external trial sites.

8.5.5 Storage and Stability

Neut™ Sodium Bicarbonate Additive Solution should be maintained at room temperature.

The release testing for Neut™ 0.4% fill includes sterility, bacteriostasis and fungistasis, endotoxin, pH and particulate testing. The testing will be performed until the end of the study.

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

See section 8.1.

8.6 Preparation of CMVPepVax

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

Preparation of CMVPepVax using the initial lot of Tet-CMV (Lot # L0402009) are detailed in Appendix F.

Note: the CMVPepVax vaccine must be administered within 90 minutes after the Tet-CMV has thawed.

Thawing and preparing CMVPepVax components (using Lot#L1501005 of Tet-CMV)

- Put cooling block in hood and set to 4°C. Once the LCD on the cooling block reads 4°C, measure the temperature of the cooling block using the NIST thermometer and "Enviro-Safe liquid', or digital thermometer. Adjust LCD until NIST or digital thermometer reads 4°C. Only the thermometer should be relied upon for accuracy. **Record** the LCD and NIST/digital temperature readings on the Vaccine Compounding Record.
- Obtain frozen Tet-CMV vial (LOT#L1501005, 3.3 mg/mL, 1.2 mL) from the -70C freezer; **record** the time at which the Tet-CMV vial is removed from the freezer. Allow vaccine vial to thaw at room temperature (approximately 15-30 minutes). **Record** the time at which the vial is completely thawed. This is the **start time** of the CMVPepVax vaccine dose preparation. **Administration of the vaccine must occur within 90 minutes after start time of preparation.**
- Once thawed, place vial on the cooling block cooled to 4°C (based only on NIST or digital thermometer reading, not the LCD) to keep cold.
- Obtain 10mM sodium acetate, pH 4.2 vial (1.1 mL), PF-03512676 and Normal Saline from Pharmacy refrigerator. Keep sodium acetate and PF-03512676 cold by placing them on the cooling block. Preparation of vaccine must be done at approximately 4°C to minimize “clouding” of solution.

- For the PF-03512676 only, using sterile procedures, inject 0.6 ml Normal Saline solution into the PF-03512676 vial (containing a total of 23.25 mg in 1.55ml, with an extractable volume of 18 mg/1.2 ml of PF-03512676), mix thoroughly and keep on ice. The final concentration of PF-03512676 will be 10.81 mg/ml in 2.15 ml total volume.

CMVPepVax injection preparation

- Add 0.15mL of 10mM sodium acetate, PH 4.2 to the thawed vial of Tet-CMV .
- Add 0.15 ml of diluted PF-03512676 (**10.81mg/ml)** to the thawed vial of Tet-CMV + sodium acetate.
- Vortex the vial (containing 1.5 ml of Tet-CMV + sodium acetate + PF-03512676) for 30 seconds at highest setting.
- Withdraw 1 ml for the subject's dose and cap syringe. This will provide a **final dosage of 2.64 mg Tet-CMV and 1.08 mg of PF-03512676**. After labeling appropriately, place syringe in sealable plastic amber bag and place in an insulated cooler .
- **Record** the time that the CMVPepVax vaccine is placed in an insulated cooler and is ready for transport.

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

- The prepared syringe or accompanying documents should be labeled in a manner to maintain a blind randomization status with the clinical study team and participant.
- The time by which the vaccine needs to be administered – start time + 90 minutes – must be clearly noted in order to inform the person administering the vaccine (e.g. "Administer before xx:xx am/pm").

8.7 Preparation of Placebo

Note: the placebo vaccine must be administered within 90 minutes after the Neut™ vial is uncapped.

Preparation

- Obtain 10mM sodium acetate, pH 4.2 vial from Pharmacy refrigerator. Obtain Neut™ 0.4% flip top vial from RT shelf.
- **Record** the time at which the Neut™ vial is opened; this is the **start time** of the placebo dose preparation. Place vials on the cooling block.
- Add 0.4 ml of Neut™ 0.4% into the 1.1 ml sodium acetate, pH 4.2 vial.
- Vortex the vial (containing 1.5 ml of volume) for 30 seconds at highest setting.
- Withdraw 1 ml for the subject's dose and cap syringe.
- After labeling appropriately, place syringe in sealable plastic amber bag and place in an insulated cooler. **Record** the time that the placebo is placed in insulated cooler and is ready for transport.

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

- The prepared syringe or accompanying documents should be labeled in a manner to maintain a blind randomization status with the clinical study team and participant.
- The time by which the placebo needs to be administered – start time + 90 minutes – must be clearly noted in order to inform the person administering the vaccine (e.g. "Administer before xx:xx am/pm").

9.0 CORRELATIVE/SPECIAL STUDIES

9.1 Immunogenicity testing

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

9.1.1 Specimen Collection and Transport to Processing Laboratory

All participants will undergo serial blood sampling for immunogenicity testing at the schedule indicated in the Study Activity Calendar (Section 10), which, in general, includes days 28, 42, 56, 70, 84, 100, 140, 180, 270 and 365 post-HCT.

Approximately 30 ml of blood in heparin (green-top) tubes, gently inverted several times to mix anti-coagulant, will be kept at room temperature and transported to authorized personnel of the Department of Experimental Therapeutics (DET) at COH or an assigned Laboratory at external trial sites. Where sample processing at an external site is not possible, whole blood will be shipped at room temperature to COH for processing. Patient name, MRN and date of birth should be defaced on the blood tube label. UPN and visit date only for the sample should be recorded on a sheet of paper sent with the blood draw.

9.1.2 Initial specimen processing, storage, and shipping

Sample processing and storage will occur in Fox South, 1002 or the external trial sites. 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, and cryopreserved in a centrally monitored liquid nitrogen tanks at the COH DET and or external trial sites . Samples will be processed and stored according to institutional SOP; it is recommended to freeze 5 million cells per aliquot. Samples will be labeled with the study timepoint (e.g. Day 42), date of collection, and study protocol number (COH 13494) and participant study number (e.g. UMN-006).

*During the processing of the PBMCs, 3 mls of plasma will be set aside, aliquoted and stored at -70C.

When requested by the laboratory performing the analysis, samples will be batch shipped to COH overnight on dry ice.

9.1.3 Analytical Method

CMV-specific $CD8^+$ T cells

Immunogenicity studies of CMV-specific CD8+ T cells will be performed at the COH DET and will include immune-monitoring of levels of $CD8^+$ T cells binding to A2-CMV-dextramers® (Immudex, Copenhagen, Denmark) and PD-1 expression on pp65-specific T cells, poly functionality and phenotype assessment of CMV-specific T cells[8, 10, 67]. Additionally, CMV-specific T cell growth kinetics and early cell death we be monitored. The proposed Phase II blinded placebo controlled study will definitively assess the level of association between reduced CMV reactivation and CMV pp65 specific T cells.

Multicolor FACS analyses will be used to assess poly functionality and phenotype of CMV-specific T cells[8, 10, 67]. All immune-monitoring studies will be performed by flow cytometry techniques using the Gallios ™ with Kaluza software (Beckman Coulter Inc, Brea CA).

All immunophenotyping will be conducted on freshly thawed PBMC without cultivation or stimulation in vitro. PBMC will be stained with each fluorochrome-conjugated antibody combination using standard methods with commercially fluoresceinated antibodies (BD Biosciences, San Jose, CA), as described in our published studies [93, 94]. Briefly, PBMC will be stained with either CMV $pp65_{495-503}$ or HIVgag_{77-85} (as control) APC-conjugated dextramers® and antibodies against CD3, CD8 and PD-1 labeled with APC-Cy7, FITC and PE, respectively (BD Biosciences, San Jose, CA). PBMC will be analyzed for levels of $CD8^+$ T cells binding to the dextramers and PD-1 expression by FACS. In combination with CBC, we will be able to calculate the absolute number of $pp65_{495-503}$–specific $CD8^+$ T cells/L.

Assessment of CMV-specific T cell growth kinetics will be performed using the carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution method for cell division tracking as previously detailed [95]. Briefly, proliferation will be analyzed using the CFSE dilution method, according to the manufacturer's procedure (Molecular Probes, Carlsbad, CA). To assess CD8 T cell proliferation, PBMC will CFSE labeled and incubated for 6 days with 1 µg/ml $pp65_{495-503}$ peptide or DMSO diluent as control, in the presence of anti-CD49b and CD28 (1 µg/ml). Cells will be then washed and co-stained with anti-CD8 (all antibodies from BD Biosciences) and FACS analyzed for CFSE fluorescence levels. Early cell death will be determined using the BD ApoAlert annexin V-FITC Apoptosis Kit (BD Biosciences) according to the manufacturer's instructions, and cells will be analyzed by FACS [89].

Natural Killer cell phenotype and function

Assessment of NK phenotype and function (cytotoxicity and cytokine production) will be performed at UMN TLL. These studies will determine whether vaccination of HLA A*0201 allogeneic HCT-R^+ induces adaptive NK cell population changes, and increase in the highly cytotoxic memory $NKG2C^+$ NK cells, linked to CMV reactivation, critical for CMV adaptive immune response and potentially linked to relapse reduction[17-20]. Since in the pilot Phase Ib, CMVPepVax appeared to reduce reactivation, cGVHD and relapse we will assess whether vaccinated patients have increased levels of this potent subset of NK cells[18-20]. The NK analyses will be performed at a single cell level by using a 9-color flow approach[18]. COH will provide aliquots of patient PBMC for this analysis.

GVHD Biomarkers

In the phase Ib trial of CMVPepVax in HCT recipients (COH IRB 12022) CMVPepVax administration was associated with reduced risk of chronic GVHD while there was no increase in acute GVHD. To further investigate potential immunologic impacts of CMVPepVax on GVHD, the following GVHD biomarkers will be evaluated in Fox South, 1002.

- interleukin-2 receptor α (IL2Ra), tumor necrosis factor receptor 1 (TNFR1), hepatocyte growth factor (HGF), interleukin 6 (IL6) and interleukin-8 (IL8) for systemic GVHD[76, 77]
- elafin for skin GVHD[78]
- regenerating islet-derived 3α (REG3α) for gastrointestinal GVHD[79]
- suppression of tumorigenicity 2 (ST2) for steroid-refractoriness [80]
- CXCL9 and B cell–activating factor (BAFF) for chronic GVHD[81, 82]

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

10.0 STUDY CALENDAR

Table 10.0 Study Activity Calendar

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

HCT Day 0

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

a. Study day is defined relative to the day of HCT which is defined as Day 0.
b. Window for post-HCT Day 28 and 56 vaccinations and Bi-Weekly Assessment procedures is the assigned day +/- 7 days.
c. Windows for the 'Post-100 Day Follow-Up' visits is the assigned day +/- 15 days.
d. All assessments to be performed and reviewed on the day of and prior to vaccine administration, except for CMV qPCR for which the result may remain pending.
e. On Day 70 post-HCT, 14 days after the second vaccination, administration of other vaccines is no longer prohibited (Section 5.7).
f. Informed consent process to be fully documented: e.g. prospective participant had sufficient time for deliberation, all questions were answered, treatment options provided by MD, full study

reviewed including risks, and a copy of signed consent given to participant. Informed consent must occur prior to any research only (non-SOC) screening procedures.

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

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

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

j. Concurrent medication data collected in CRFs pertains to anti-viral medications (including start and stop date), immunosuppressive agents, daily prednisone dose for the 7 days prior to vaccine administration, and prohibited medications (Section 5.7).

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

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

m. KPS scale is found in Appendix D.

n. Adverse events (AEs) will be assessed and documented from Day 28 to Day 100 at the defined study visits and at standard of care visits. Documentation should support adverse events recorded in the CRFs: all AEs considered possibly, probably or definitely related to study agent, all grade 3/4/5 events, and all serious adverse events. See Section 7.0 for adverse event reporting.

o. Engraftment status should be documented at each study visit, and if engraftment failure occurs the date of engraftment failure should be noted (see Section 5.4 for definitions).

p. See Section 5.4.4. for definition of relapse. Disease relapse will be assessed according to and per the timing of institutional SOC practice for the participant's specific hematologic malignancy. **Note:** participants who undergo disease relapse will cease all future study visits/procedures and will be followed only for survival through Day 365.

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

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

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

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

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

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

w. CMV qPCR will be performed per institutional SOC (usually twice-weekly, and at minimum once per calendar week) between days 21 and day 100 post-HCT. All CMV qPCR results will be collected in the case report forms. Post Day 100 until day 180, CMV qPCR to be performed at least every 2 weeks. All CMV qPCR results will be collected in the case file.

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

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

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

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

bb. Research blood samples are to be collected at the same time as the CBC with differential. A volume of approximately 30 ml of blood in heparin (green-top) tubes, gently inverted several times to mix anti-coagulant, and then kept at room temperature until transport to authorized personnel at Fox South, 1002 or external site Research Laboratory. 27 ml and 3 ml are allocated for PBMC and plasma isolation respectively.

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

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

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

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

gg. Vaccine administration is detailed in Section 5.3.

11.0 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

The primary endpoint of this trial will be CMV-related. Secondary endpoints will include safety, immunological and clinical parameters. In detail:

Primary endpoints:

1. For the entire cohort (n=97): Immunological endpoints: levels of $CD8^+$ T cells binding to A2-CMV-dextramers®, combined with T and NK cell phenotype and function.
2. For the combination cohort (n=36): CMV reactivation [≥1,250 IU/mL] or CMV disease between day 56 and day 180 post-HCT in patients who receive standard Prevymis prophylaxis (from day 14 through day 100).

Secondary endpoints:

1. Key safety endpoints: non-relapse mortality (NRM) at 100 days post HCT, severe (grade 3-4) aGVHD, and grade 3-4 AEs (CTCAE 4.0) probably or definitely related to the vaccination within 2 weeks from each vaccination.
2. CMV reactivation [≥1,250 IU/mL] or CMV disease prior to day 100 post-HCT. Timing and recurrence of events are included in the primary analysis.
3. CMV-related endpoints: Duration of viremia, incidence of late CMV viremia (>100 and ≤ 360 days post HCT), use of antiviral drugs (triggered by clinically significant viremia), cumulative number of CMV specific antiviral treatment days.
4. Clinical endpoints: time to engraftment, incidence of aGVHD, chronic GVHD (cGVHD), relapse, non-relapse mortality, all-cause mortality, non-CMV infections.
5. Immunologic endpoints for the combination cohort (n=36): Immune reconstitution measured as levels, function and kinetics of CMV-specific T cells through day 180 post-HCT in patients who receive standard Prevymis prophylaxis (day 14 through day 100).

12.0 STATISTICAL CONSIDERATIONS

12.1 Study Design

This is a randomized, blinded and placebo controlled Phase II trial conducted at multiple centers, with data coordination at COH. After the trial met its futility stopping criterion, the trial has been amended to include a cohort of patients on Prevymis prophylaxis. This constitutes a major change of the clinical setting, with Prevymis prophylaxis required for eligibility. Because 7 of the 8 primary outcome events in the initial trial occurred prior to the second CMVPepVax injection, the change in eligibility is thought to be directly relevant to the reason for the futility outcome. The primary aims are given in the previous section 1, and include evaluation of cellular immunity both in general, via stratified analysis, and in the Prevymis cohort as a subgroup. Evaluation of CMV events will expose the therapeutic concept to a futility assessment, a strategy that often motivates expansion cohorts in early-phase trials. The study includes rules for periodic safety monitoring, but futility assessment now applies to the full CMVPepVax arm of the Prevymis cohort. and an interim analysis for futility.

Update at the time of amendment

Enrollment was stopped when 48 subjects had been followed for 100 days, because 8 of the subjects with reactivation events by d100, more than half (5 of the 8) were on the vaccine arm, these events triggered the futility stopping rule. The combined reactivation rate at this point was 8/48, or 17%, very close to the 15% rate that was expected for the vaccine arm, but much lower than expected for the placebo arm. At such low reactivation rates, the futility rule has a much higher false alarm rate that planned. If the vaccine offers the same degree of protection originally planned for (i.e. preventing 25 of 40 reactivations) the futility rule would have a false alarm rate of approximately 27%. The interim result is thus not inconsistent with the original targeted effect size, and does not provide a satisfactory

contradictory signal to the success seen in protocol 12022. The interim result do, however, indicate the likely futility of the original primary analysis, and the problem of reactivations occurring too early to complete CMVPepVax administration may be avoided in the context of Prevymis prophylaxis. The amended protocol abandons the hypothesis test on the original primary endpoint in favor of estimating and characterizing the incidence and timing of CMV reactivation when CMVPepVax is used in combination with Prevymis prophylaxis. This will inform further development both by subjecting CMVPepVax to a futility evaluation with regard to the relative incidence of CMV events, and by providing novel longitudinal measurements of immune function in response to the vaccine while CMV load is suppressed by Prevymis.

12.2 Randomization

The trial has a target of 97 randomized HCT recipients. Eligible HCT-R+ will be consented and enrolled pre-HCT, and registered through COH DCC. A computer-generated randomization program, stratified by donor CMV serostatus will assign registered participants to the CMVPepVax or placebo arm; the treatment assignment will be generated and provided to site pharmacists, who are unmasked to treatment-group allocation, in advance of planned vaccination. Registered participants will be followed for the course of transplant and be assessed for the eligibility for 'Day 28 post-HCT' vaccination.

Participants who meet the initial vaccine administration criteria will receive an injection; participants failing to meet the criteria will discontinue study participation and be replaced. Only participants who receive an injection will be considered "randomized". Information regarding registered participants who do not receive vaccine will be entered into the computer-generated randomization program to inform subsequent treatment assignments. Participants who do not meet the criteria to receive the first vaccine (CMVPepVax or placebo) will leave the study, without further treatment or follow-up, but their data collected up to study departure will be included in the trial dataset as non-randomized subjects, and these data will be incorporated into estimates of event rates post-HCT based on the intention to treat.

12.3 Sample Size Accrual Rate

This trial was originally planned to randomize 97 HCT-R+, in a 1:1 ratio, to either the CMVPepVax vaccine arm (N=48 or 49), or to the placebo arm (N=49 or 48). The amended trial will use the same randomization procedures to assign the final 36 subjects, 18 to each arm. Each patient in the new cohort (36 patients, 18 vs 18) will receive Prevymis as standard of care. The uncertainty in the per-arm totals is due to continued masking at the time of amendment. With the expected dropout rate of 10-15% prior to randomization, the total accrual is expected to be 106-115. Over the past five years, COH, UMN, FHCRC, OSUMC, and Emory University combined have performed on-average ~500 adult allogeneic HCT procedures annually. We anticipate about 70 to be eligible annually being HLA A*0201 and CMV seropositive. 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.

Planning for measures of immune function is based on results of COH protocol 12022, the open-label analog of this placebo-controlled protocol. In that trial, 10 of 18 vaccinated subjects developed CMV pp65-specific CD8+ T-cells that were strongly concentrated (> 80%) in effector memory phenotypes, and did so without CMV reactivation. This can be compared to 1 of 14 non-vaccinated subjects who did not reactivate. In the current study, the planned sample size of 48 subjects per arm would provide approximately 80% power for a one-sided 0.05-level test assuming a 50% rate of this event from a 25% rate on the placebo arm. Beyond this measure of vaccine-induced immune function, the data will be used to describe the longitudinal changes in immune function with and without vaccine, in subjects who

do and do not reactivate CMV, to serve as a reference for comparison of future immune function data which will include patients receiving Prevymis as prophylaxis.

With regard to the incidence of CMV events, the sample size of 48 individuals per arm was originally designed to detect a reduction in CMV reactivation from 40% to 15% at 100 days post-HCT, providing 90% power at one-sided 10% significance level (these are the typical operational characteristics in randomized Phase II trials [21]). The aim of this hypothesis test has been abandoned after the interim analysis and substituted with a preliminary analysis of patients receiving Prevymis prophylaxis. For the Prevymis cohort the 36 patients will be randomized 1:1 (18/18) to receive CMVPepVax or Placebo. Based on a Phase III trial of Prevymis, we expect 17 percent of placebo subjects to reactivate after second vaccination on week 8. This is based on the expectation that 33 percent will be high-risk subjects. Assuming that CMVPepVax vaccination during prophylaxis can reduce the week 8 through week 26 reactivation rate to 5 percent, the probability that there are more reactivations on the placebo arm than on the CMVPepVax arm is approximately 88 percent, which can be regarded as the power for a non-futile finding. If CMVPepVax offers no protection, there would be a 50 percent chance of a futility finding, and T-cell data would likely indicate many subjects with poor CMVPepVax antigen recognition. If CMVPepvax has strong protection (5% reactivation), a finding of futility is unlikely (12 percent) and an evident biological effect would be expected in most patients.

12.4 Data Analysis Plan

Cellular immunity

The data analysis for the first primary objective is the secondary analysis of aim of estimating the effect of vaccination on cellular immunity in the full set of approximately 97 subjects. This will necessarily be exploratory in nature, because of the multivariate and longitudinal nature of cellular immunity. The information gained may enable the construction of a more detailed analysis, capable of a priori registration in a subsequent trial. A comparison across arms of the number of subjects who achieve an elevated concentration of antigen-specific T-cells in the TEM and TEMRA category, stratified by Prevymis usage, will serve as the pre-specified primary analysis for this objective. The estimated power for this analysis is given in section 12.3. The same analysis will also be applied to the Prevymis cohort as a subgroup. The longitudinal CMV-specific cellular assay data will be modeled on a logarithmic scale, using a generalized estimating equation approach to accommodate the stochastic dependence through time. This produces an estimated multiplicative effect of vaccination, qualified by a valid estimate of variability.

Clinical Efficacy

The primary analysis of efficacy in the amended trial is restricted to the Prevymis cohort. Each randomized study subject will be followed according to the study calendar (section 11) for the occurrence of CMV reactivation events, which are defined in section 11 in terms of either viremia, low-level viremia treated with antivirals, or CMV disease. Both initial and recurrent events will be recorded, with patients considered at risk for recurrent events after completion of a full planned course of anti-viral therapy. If PCR results from blood drawn on the day of first injection should indicate reactivation, the subject will be replaced and excluded from the primary comparison, but the subject will, in all other respects, continue on the study with all planned treatments and data collections. The primary analysis will focus on arm-specific counts of subjects with events in the defined period, with binomial confidence intervals, and comparison by Fisher's exact test. In addition, vaccine and placebo groups will be compared with regard to (a) the hazard of CMV events, using time to event models; and (b) the total days of CMV-directed antiviral therapy per individual, using the Mann-Whitney statistic.

Futility

An interim analysis for futility was conducted when the first 48 randomized subjects were evaluable for the primary endpoint (i.e. had a CMV event or reached 100 days without a CMV event). Among the 36 subjects planned for the Prevymis cohort, the incidence of more CMV events on the CMVPepVax arm than the placebo arm would constitute a futility signal, so there will be no separate interim analysis for futility.

12.5 Safety Monitoring

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

Monitoring of the Prevymis cohort will be done conservatively as both a continuation of the original monitoring plan, and as a restart of that same monitoring plan applied to the new patients receiving CMVPepVax in combination with Prevymis.

Specifically:

(1) 100 days non-relapse mortality (NRM) will be monitored as the 12^{th}, 24^{th} and 36^{th} subject on the vaccine arm reaches the 100 day evaluation point. Operationally, the CRA will notify the monitoring statistician as cohorts of 24 patients (12 vaccinated) near the 100 day mark. If NRM frequencies exceed 4, 6, or 8, at the designated 100 day evaluation point, then the trial will be suspended for safety review by the COH and external site DSMCs. These numbers were selected to limit the overall false-alarm probability for this endpoint to less than 0.02 when there is no additional risk due to immunization and were based on a benchmark risk of 10%.

(2) Severe acute GVHD (aGVHD, grade 3-4) will be monitored as every 12^{th} subject on the vaccine arm reaches the 100 day evaluation point. The trial will be interrupted if 6 or more of the first 12 recipients, or 9 of 24, or 11 of 36, experience Grade 3-4 aGVHD. This would be a significant elevation from the COH/UMN historical benchmark of 15% of allogeneic HCT recipients with matched sibling donors[23]. These rules are determined to limit the overall aGVHD false alarm probability to 0.02 if vaccination does not increase risk[23].

(3) Any serious AEs (SAE, grade 3-4) related to the vaccination within 2 weeks from each vaccination will be reviewed by the PMT when each 12^{th} patient on the CMVPepVax arm passes the 2 week post-injection time for their second injection.

13.0 DATA HANDLING, DATA MANAGEMENT, RECORD KEEPING

13.1 Source Documents

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

13.2 Data Capture Methods and Management

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

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

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

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

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

13.3 Case Report Forms/Data Submission Schedule

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

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

All data will be collected using electronic data collection system described in Section 13.2, and will be submitted according to the timelines indicated in Table 13.1.3.

Table 13.3 Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 14 calendar days of treatment administration
Adverse Event Report Forms	Within 14 calendar days of the study visit
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms	Within 10 calendar days of the assessment

(concomitant medications, chemistry, hematology etc.)	
Off Treatment/Off Study Forms	Within 10 calendar days of completing treatment or being taken off study for any reason

13.4 Regulatory Records

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

14.0 ADHERENCE TO THE PROTOCOL

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

All deviations from the protocol must be documented in study subject source documents and promptly reported to the Study PI and to the local IRB according to its policies requirements. The Study PI will report the deviation according to City of Hope's deviation policy for reporting deviations.

14.1.1 Emergency Modifications

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

For any such emergency modification implemented,

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

14.1.2 Planned Non-Emergency Deviations

All non-emergency planned deviations from the protocol must have **prior** approval by the Study Principal Investigator, the Site Principal Investigator and the local IRB, and if applicable the COH IRB Unplanned Deviations – Deviations Discovered After They Have Occurred

For deviations to the protocol discovered after they have occurred,

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

A list of deviations from all participating sites will be submitted along with the Protocol Management Team (PMT) progress report to the COH DSMC.

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

15.1 Site Principal Investigator

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

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events, deviations, and unanticipated problems.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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

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

15.2 Study Principal Investigator

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

15.3 Protocol Management Team (PMT)

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

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

15.4 Monitoring

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

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

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

Staff from OCTAM will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the City of Hope OCTAM SOP document. Documentation of monitoring activities and findings will be provided to the site study team, the site PI, study PI, and the COH DSMC.

15.5 Quality Assurance

The City of Hope Clinical Research Information Support will provide quality assurance as detailed in the COH DCC Operations Plan provided as a supplement to this document.

15.6 City of Hope Data and Safety Monitoring Committee

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

The DSMC is a multidisciplinary committee charged with overseeing the monitoring of safety of participants in clinical trials, and the conduct, progress, validity, and integrity of the data for all clinical trials that are sponsored by City of Hope. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. The committee reviews the

progress and safety of all active research protocols that are not monitored by another safety and data monitoring committee or board.

The COH Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. Information that raises any questions about participant safety will be addressed with the Principal Investigator, statistician and study team. The COH DSMC Charter is a supplement to this protocol.

The DSMC will review the study's status quarterly and/or more often if necessary. The DSMC will review up-to-date participant accrual; summary of all adverse events captured via routine and expedited reporting; a summary of deviations; any response information; monitoring reports, and summary comments provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request. A review of outcome results (response, toxicity and adverse events) and factors external to the study (such as scientific or therapeutic developments) is discussed, and the Committee votes on the status of each study.

Data and safety will be reported to the COH DSMC using the PMT report and submitted quarterly from the date of activation.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Ethical Standard

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

16.2 Regulatory Compliance

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

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

16.3 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate IRB holding a current US Federalwide 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, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent document will be in the possession of the Investigator, and, for sites external to COH, the possession of the coordinating center, before the study is initiated. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

The IRB will be informed of revisions to other documents originally submitted for review; serious unexpected or unanticipated adverse experiences occurring during the study, and any additional adverse experiences in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

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

16.4 Informed Consent

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

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

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

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

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

16.5.1 Inclusion of Women and Minorities

The study is open anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue and randomize approximately 97 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

Women who are pregnant or plan to become pregnant are excluded from participation because PF-03512676, the adjuvant used in CMVPepVax, has been determined to be embryolethal and teratogenic in animal testing, and as such, CMVPepVax has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the administered vaccine, breastfeeding should be discontinued if the mother is enrolled on this study.

16.5.2 Exclusion of Pediatric Patients

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

16.5.3 Exclusion of HIV Positive Individuals

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

16.6 Participant Confidentiality

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

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

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

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

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

16.7 Conflict of Interest

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

16.8 Financial Obligations, Compensation, and Reimbursement of Participants

The investigational drug including Tet-CMV diluted in Na acetate and co-injected with PF-03512676 adjuvant, will be provided free of charge by NCI and Pfizer, respectively.

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

The standard of care drugs or procedures provided during the course of study participation will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study.

In the event of physical injury to a research participant resulting from research procedures, appropriate medical treatment will be available at City of Hope to the injured research participant. There are no plans for City of Hope to provide financial compensation in the event of physical injury to a research participant.

The research participant will not receive reimbursement or payment for taking part in this study.

16.9 Publication/Data Sharing

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by City of Hope for the purposes of performing the study, will be published or passed on to any third party without the written approval of Dr. Don Diamond. Any investigator involved with this study is obligated to provide City of Hope with complete test results and all data derived from the study.

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the City of Hope and

participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

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

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

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

17.0 REFERENCES

1. Applebaum, F.R., et al., *Nonbacterial nonfungal pneumonia following marrow transplantation in 100 identical twins.* Transplantation, 1982. **33**(3): p. 265-8.
2. Quinnan, G.V., Jr., et al., *Cytotoxic T cells in cytomegalovirus infection: HLA-restricted T- lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone-marrow- transplant recipients.* N.Engl.J.Med., 1982. **307**: p. 7-13.
3. Reusser, P., et al., *Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation.* Blood, 2002. **99**(4): p. 1159-1164.
4. Winston, D.J., et al., *Infectious complications of human bone marrow transplantation.* Medicine (Baltimore), 1979. **58**(1): p. 1-31.
5. Boeckh, M. and W.G. Nichols, *The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy.* Blood, 2004. **103**(6): p. 2003-2008.
6. Nichols, W.G., et al., *High Risk of Death Due to Bacterial and Fungal Infection among Cytomegalovirus (CMV)-Seronegative Recipients of Stem Cell Transplants from Seropositive Donors: Evidence for Indirect Effects of Primary CMV Infection.* J.Infect.Dis., 2002. **185**(3): p. 273-282.
7. La Rosa, C., et al., *Preclinical development of an adjuvant-free peptide vaccine with activity against CMV pp65 in HLA transgenic mice.* Blood, 2002. **100**(10): p. 3681-3689.
8. La Rosa, C., et al., *Clinical evaluation of safety and immunogenicity of PADRE-cytomegalovirus (CMV) and Tetanus-CMV fusion peptide vaccines with or without PF03512676 adjuvant.* J.Infect.Dis., 2012. **205**(8): p. 1294-1304.
9. Sylwester, A.W., et al., *Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects.* J.Exp.Med., 2005. **202**(5): p. 673-685.
10. Gratama, J.W., et al., *Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study.* Blood, 2010. **116**(10): p. 1655-1662.
11. Marty, F.M., et al., *Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation.* N Engl J Med, 2017. **377**(25): p. 2433-2444.
12. Khanna, R. and D.J. Diamond, *Human cytomegalovirus vaccine: time to look for alternative options.* Trends Mol.Med., 2006. **12**(1): p. 26-33.
13. Aubert, G., et al., *Cytomegalovirus-specific cellular immune responses and viremia in recipients of allogeneic stem cell transplants.* J.Infect.Dis., 2001. **184**(8): p. 955-963.
14. Wieand, S., G. Schroeder, and J.R. O'Fallon, *Stopping when the experimental regimen does not appear to help.* Stat.Med., 1994. **13**(13-14): p. 1453-1458.
15. Lacey, S.F., et al., *Characterization of cytotoxic function of CMV-pp65-specific CD8+ T- lymphocytes identified by HLA tetramers in recipients and donors of stem-cell transplants.* Transplantation, 2002. **74**(5): p. 722-732.
16. Villacres, M.C., et al., *Human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy maintain activated cd8+ t cell subsets as a strong adaptive immune response to cytomegalovirus.* J.Infect.Dis., 2001. **184**(3): p. 256-267.
17. Foley, B., et al., *Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand in vivo in response to recipient CMV antigen.* J.Immunol., 2012. **189**(10): p. 5082-5088.
18. Foley, B., et al., *Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function.* Blood, 2012. **119**(11): p. 2665-2674.
19. Lopez-Verges, S., et al., *Expansion of a unique CD57(+)NKG2Chi natural killer cell subset during acute human cytomegalovirus infection.* Proc.Natl.Acad.Sci.U.S.A, 2011. **108**(36): p. 14725-14732.
20. Kuijpers, T.W., et al., *Human NK cells can control CMV infection in the absence of T cells.* Blood, 2008. **112**(3): p. 914-915.

21. Rubinstein, L.V., et al., *Design issues of randomized phase II trials and a proposal for phase II screening trials.* J.Clin.Oncol., 2005. **23**(28): p. 7199-7206.

22. Przepiorka, D., et al., *1994 Consensus Conference on Acute GVHD Grading.* Bone Marrow Transplant, 1995. **15**(6): p. 825-8.

23. Bensinger, W.I., et al., *Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers.* N.Engl.J.Med., 2001. **344**(3): p. 175-181.

24. Steininger, C., *Clinical relevance of cytomegalovirus infection in patients with disorders of the immune system.* Clin.Microbiol.Infect., 2007. **13**(10): p. 953-963.

25. Limaye, A.P., et al., *Impact of cytomegalovirus in organ transplant recipients in the era of antiviral prophylaxis.* Transplantation, 2006. **81**(12): p. 1645-1652.

26. Griffiths, P., et al., *Contemporary management of cytomegalovirus infection in transplant recipients: guidelines from an IHMF workshop, 2007.* Herpes., 2008. **15**(1): p. 4-12.

27. Boeckh, M., et al., *Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies.* Biol.Blood Marrow Transplant., 2003. **9**(9): p. 543-558.

28. Tomblyn, M., et al., *Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective.* Biol.Blood Marrow Transplant, 2009. **15**(10): p. 1143-1238.

29. Zerr, D.M., et al., *HHV-6 reactivation and its effect on delirium and cognitive functioning in hematopoietic cell transplantation recipients.* Blood, 2011. **117**(19): p. 5243-5249.

30. Reusser, P., *Oral valganciclovir: a new option for treatment of cytomegalovirus infection and disease in immunocompromised hosts.* Expert.Opin.Investig.Drugs, 2001. **10**(9): p. 1745-1753.

31. Ljungman, P., M. Hakki, and M. Boeckh, *Cytomegalovirus in hematopoietic stem cell transplant recipients.* Infect.Dis.Clin.North Am., 2010. **24**(2): p. 319-337.

32. Hakki, M., et al., *Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: impact of host factors, drug therapy, and subclinical reactivation.* Blood, 2003. **102**(8): p. 3060-3067.

33. Nguyen, Q., et al., *Late cytomegalovirus pneumonia in adult allogeneic blood and marrow transplant recipients.* Clin.Infect.Dis., 1999. **28**(3): p. 618-623.

34. Razonable, R.R., et al., *Allograft rejection predicts the occurrence of late-onset cytomegalovirus (CMV) disease among CMV-mismatched solid organ transplant patients receiving prophylaxis with oral ganciclovir.* J.Infect.Dis., 2001. **184**(11): p. 1461-1464.

35. Boeckh, M., et al., *Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity.* Blood, 2003. **101**(2): p. 407-414.

36. Peggs, K.S. and S. Mackinnon, *Immune reconstitution following haematopoietic stem cell transplantation.* Br.J.Haematol., 2004. **124**(4): p. 407-420.

37. Singh, N., *Delayed occurrence of cytomegalovirus disease in organ transplant recipients receiving antiviral prophylaxis: are we winning the battle only to lose the war?* Eur.J.Clin.Microbiol.Infect.Dis., 2002. **21**(12): p. 902.

38. Gamadia, L.E., et al., *Primary immune responses to human CMV: a critical role for IFN-gamma -producing CD4+ T cells in protection against CMV disease.* Blood, 2003. **101**(7): p. 2686-2692.

39. Diamond, D.J., et al., *Development of a candidate HLA A*0201 restricted peptide-based vaccine against human cytomegalovirus infection.* Blood, 1997. **90**(5): p. 1751-1767.

40. La Rosa, C., et al., *Enhanced immune activity of cytotoxic T-lymphocyte epitope analogs derived from positional scanning synthetic combinatorial libraries.* Blood, 2001. **97**(6): p. 1776-1786.

41. Wills, M.R., et al., *The human CTL response to cytomegalovirus is dominanted by structural protein pp65: Frequency, specificity, and T Cell Receptor usage of pp65-Specific CTL.* J.Virol., 1996. **70**(11): p. 7569-7579.

42. Riddell, S.R., et al., *Class I MHC-restricted cytotoxic T lymphocyte recognition of cells infected with human cytomegalovirus does not require endogenous viral gene expression.* J.Immunol., 1991. **146**(8): p. 2795-2804.

43. Bernstein, D.I., et al., *Randomized, double-blind, Phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers.* Vaccine, 2009. **28**(2): p. 484-493.

44. Wloch, M.K., et al., *Safety and immunogenicity of a bivalent cytomegalovirus DNA vaccine in healthy adult subjects.* J.Infect.Dis., 2008. **197**(12): p. 1634-1642.

45. Berencsi, K., et al., *A canarypox vector-expressing cytomegalovirus (cmv) phosphoprotein 65 induces long-lasting cytotoxic t cell responses in human cmv- seronegative subjects.* J.Infect.Dis., 2001. **183**(8): p. 1171-1179.

46. Reusser, P., et al., *Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease.* Blood, 1991. **78**(5): p. 1373-1380.

47. Rook, A.H., et al., *Importance of cytotoxic lymphocytes during cytomegalovirus infection in renal transplant recipients.* Am.J.Med., 1984. **76**: p. 385-392.

48. Cobbold, M., et al., *Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers.* J.Exp.Med., 2005. **202**(3): p. 379-386.

49. Walter, E.A., et al., *Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor.* N.Engl.J.Med., 1995. **333**: p. 1038-1044.

50. Feuchtinger, T., et al., *Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation.* Blood, 2010. **116**(20): p. 4360-4367.

51. Einsele, H., et al., *Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy.* Blood, 2002. **99**(11): p. 3916-3922.

52. Peggs, K.S., et al., *Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines.* Lancet, 2003. **362**(9393): p. 1375-1377.

53. Cwynarski, K., et al., *Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation.* Blood, 2001. **97**(5): p. 1232-1240.

54. Gallez-Hawkins, G., et al., *Cytomegalovirus immune reconstitution occurs in recipients of allogeneic hematopoietic cell transplants irrespective of detectable cytomegalovirus infection.* Biol.Blood Marrow Transplant., 2005. **11**(11): p. 890-902.

55. Gratama, J.W., et al., *Tetramer-based quantification of cytomegalovirus (CMV)-specific CD8+ T lymphocytes in T-cell-depleted stem cell grafts and after transplantation may identify patients at risk for progressive CMV infection.* Blood, 2001. **98**(5): p. 1358-1364.

56. Einsele, H. and H. Hebart, *Cellular immunity to viral and fungal antigens after stem cell transplantation.* Curr.Opin.Hematol., 2002. **9**(6): p. 485-489.

57. Longmate, J., et al., *Population coverage by HLA class-I restricted cytotoxic T-lymphocyte epitopes.* Immunogenetics, 2001. **52**(3-4): p. 165-173.

58. Zaia, J.A., et al., *Infrequent Occurrence of Natural Mutations in the pp65(495-503) Epitope Sequence Presented by the HLA A*0201 Allele among Human Cytomegalovirus Isolates.* J.Virol., 2001. **75**(5): p. 2472-2474.

59. Mocarski, E.S., Jr., *Immunomodulation by cytomegaloviruses: manipulative strategies beyond evasion.* Trends Microbiol., 2002. **10**(7): p. 332-339.

60. Adler, S.P., et al., *Safety and immunogenicity of the Towne strain cytomegalovirus vaccine.* Pediatr.Infect.Dis.J., 1998. **17**(3): p. 200-206.

61. Alexander, J., et al., *Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides.* Immunity., 1994. **1**: p. 751-761.

62. Panina-Bordignon, P., et al., *Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells.* Eur.J.Immunol., 1989. **19**(12): p. 2237-2242.

63. Krieg, A.M., *CpG motifs in bacterial DNA and their immune effects.* Annu.Rev.Immunol., 2002. **20**: p. 709-760.

64. Rothenfusser, S., et al., *CpG-A and CpG-B oligonucleotides differentially enhance human peptide-specific primary and memory CD8+ T-cell responses in vitro.* Blood, 2004. **103**(6): p. 2162-2169.

65. Cooper, C.L., et al., *CPG 7909 adjuvant improves hepatitis B virus vaccine seroprotection in antiretroviral-treated HIV-infected adults.* AIDS, 2005. **19**(14): p. 1473-1479.

66. Hartmann, G. and A.M. Krieg, *Mechanism and function of a newly identified CpG DNA motif in human primary B cells.* J.Immunol., 2000. **164**(2): p. 944-953.

67. Zhou, W., et al., *Impact of donor CMV status on viral infection and reconstitution of multifunction CMV-specific T cells in CMV-positive transplant recipients.* Blood, 2009. **113**(25): p. 6465-6476.

68. Davila, E., et al., *Creating space: an antigen-independent, CpG-induced peripheral expansion of naive and memory T lymphocytes in a full T-cell compartment.* Blood, 2002. **100**(7): p. 2537-2545.

69. Kuo, C.C., S.M. Liang, and C.M. Liang, *CpG-B oligodeoxynucleotide promotes cell survival via up-regulation of Hsp70 to increase Bcl-xL and to decrease apoptosis-inducing factor translocation.* J.Biol.Chem., 2006. **281**(50): p. 38200-38207.

70. Woolfrey, A., et al., *HLA-C antigen mismatch is associated with worse outcome in unrelated donor peripheral blood stem cell transplantation.* Biol Blood Marrow Transplant, 2011. **17**(6): p. 885-92.

71. Saber, W., et al., *Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia.* Blood, 2012. **119**(17): p. 3908-16.

72. Bacigalupo, A., *Matched and mismatched unrelated donor transplantation: is the outcome the same as for matched sibling donor transplantation?* Hematology Am Soc Hematol Educ Program, 2012. **2012**: p. 223-9.

73. Anasetti, C., *What are the most important donor and recipient factors affecting the outcome of related and unrelated allogeneic transplantation?* Best Pract Res Clin Haematol, 2008. **21**(4): p. 691-7.

74. Sorror, M.L., et al., *Long-term outcomes among older patients following nonmyeloablative conditioning and allogeneic hematopoietic cell transplantation for advanced hematologic malignancies.* JAMA, 2011. **306**(17): p. 1874-1883.

75. Warlick, E., et al., *Reduced intensity conditioning is superior to nonmyeloablative conditioning for older chronic myelogenous leukemia patients undergoing hematopoietic cell transplant during the tyrosine kinase inhibitor era.* Blood, 2012. **119**(17): p. 4083-4090.

76. Paczesny, S., et al., *A biomarker panel for acute graft-versus-host disease.* Blood, 2009. **113**(2): p. 273-8.

77. Levine, J.E., et al., *Acute graft-versus-host disease biomarkers measured during therapy can predict treatment outcomes: a Blood and Marrow Transplant Clinical Trials Network study.* Blood, 2012. **119**(16): p. 3854-60.

78. Paczesny, S., et al., *Elafin is a biomarker of graft-versus-host disease of the skin.* Sci Transl Med, 2010. **2**(13): p. 13ra2.

79. Ferrara, J.L., et al., *Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease.* Blood, 2011. **118**(25): p. 6702-8.

80. Vander Lugt, M.T., et al., *ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death.* N Engl J Med, 2013. **369**(6): p. 529-39.

81. Kennedy, G.A., et al., *Addition of interleukin-6 inhibition with tocilizumab to standard graft-versus-host disease prophylaxis after allogeneic stem-cell transplantation: a phase 1/2 trial.* Lancet Oncol, 2014. **15**(13): p. 1451-9.

82. Sarantopoulos, S., et al., *Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease.* Blood, 2009. **113**(16): p. 3865-74.

83. Cooper, C.L., et al., *CPG 7909, an immunostimulatory TLR9 agonist oligodeoxynucleotide, as adjuvant to Engerix-B HBV vaccine in healthy adults: a double-blind phase I/II study.* J.Clin.Immunol., 2004. **24**(6): p. 693-701.

84. Vitiello, A., et al., *Development of a lipopeptide-based therapeutic vaccine to treat chronic HBV infection. I. Induction of a primary cytotoxic T lymphocyte response in humans.* J.Clin.Invest., 1995. **95**: p. 341-349.

85. Waldner, H., *The role of innate immune responses in autoimmune disease development.* Autoimmun.Rev., 2009. **8**(5): p. 400-404.

86. Maury, S., et al., *Prolonged immune deficiency following allogeneic stem cell transplantation: risk factors and complications in adult patients.* Br.J.Haematol., 2001. **115**(3): p. 630-641.

87. Storek, J., et al., *Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation.* Blood, 2001. **97**(11): p. 3380-3389.

88. Skert, C., et al., *Kinetics of Th1/Th2 cytokines and lymphocyte subsets to predict chronic GVHD after allo-SCT: results of a prospective study.* Bone Marrow Transplant., 2009. **44**(11): p. 729-737.

89. Elmaagacli, A.H., et al., *Early human cytomegalovirus replication after transplantation is associated with a decreased relapse risk: evidence for a putative virus-versus-leukemia effect in acute myeloid leukemia patients.* Blood, 2011. **118**(5): p. 1402-1412.

90. Green, M.L., et al., *CMV reactivation after allogeneic HCT and relapse risk: evidence for early protection in acute myeloid leukemia.* Blood, 2013. **122**(7): p. 1316-1324.

91. La Rosa, C., et al., *Rapid Acquisition of Cytomegalovirus-Specific T Cells with a Differentiated Phenotype, in Nonviremic Hematopoietic Stem Transplant Recipients Vaccinated with CMVPepVax.* Biol Blood Marrow Transplant, 2018.

92. Nakamura, R., et al., *Viraemia, immunogenicity, and survival outcomes of cytomegalovirus chimeric epitope vaccine supplemented with PF03512676 (CMVPepVax) in allogeneic haemopoietic stem-cell transplantation: randomised phase 1b trial.* Lancet Haematol, 2016. **3**(2): p. e87-98.

93. La Rosa, C., et al., *Longitudinal assessment of cytomegalovirus (CMV)-specific immune responses in liver transplant recipients at high risk for late CMV disease.* J.Infect.Dis., 2007. **195**(5): p. 633-644.

94. La Rosa, C., et al., *Programmed death-1 expression in liver transplant recipients as a prognostic indicator of cytomegalovirus disease.* J.Infect.Dis., 2008. **197**(1): p. 25-33.

95. Krishnan, A., et al., *Programmed death-1 receptor and interleukin-10 in liver transplant recipients at high risk for late cytomegalovirus disease.* Transpl.Infect.Dis., 2010. **12**(4): p. 363-370.