A MULTI-SITE, RANDOMIZED TRIAL OF SUBJECT-COLLECTED DRIED BLOOD SPOT CMV TESTING WITH MOBILE TECHNOLOGY SUPPORT TO OPTIMIZE PREEMPTIVE THERAPY LATE AFTER ALLOGENEIC HCT

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STATEMENT OF COMPLIANCE

This study will be carried out in compliance with the protocol and in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50, (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 812 (Investigational Device Exemptions)
- International Conference on Harmonization**:** Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements. I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

Investigator Name
Investigator Title

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LIST OF ABBREVIATIONS

AE	Adverse event
ASBMT	American Society for Blood and Marrow Transplantation
CDC	(U.S.) Centers for Disease Control and Prevention
CDS	Collaborative Data Services
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
CRF	Case report form
CRO	Contract research organization
DBS	Dried blood spots
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DMID-CROMS	(NIAID) DMID, Clinical Research Operations and Management Support
DSMB	Data and Safety Monitoring Board
EBMT	European Society for Blood and Marrow Transplantation
FDA	(U.S.) Food and Drug Administration
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GVHD	Graft versus host disease
HCT	Hematopoietic cell transplantation
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human leukocyte antigen
ICH	International Conference on Harmonisation
IDSA	Infectious Disease Society of America
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-to-treat
JAMA	Journal of the American Medical Association
LPS	Lipopolysaccharide
mITT	Modified intent-to-treat
MOP	Manual of Operations
N	Number (typically refers to subjects)
NEJM	New England Journal of Medicine

NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PCR	Polymerase chain reaction
PI	Principal Investigator
PTID	Participant identification number
qPCR	Quantitative polymerase chain reaction
SAE	Serious adverse event
SDCC	Statistical and Data Coordinating Center
SMC	Study Monitoring Committee
SOP	Standard operating procedures
TNF-α	Tumor necrosis factor alpha
USPS	United States Postal Services

PROTOCOL SUMMARY

Title:	A multi-site randomized trial of subject-collected CMV dried blood spot testing with mobile technology support to optimize preemptive therapy late after allogeneic HCT
Population:	150 allogeneic hematopoietic cell transplantation (HCT) recipients ≥15 years of age, who are considered by their transplant team to be at risk for late cytomegalovirus (CMV) disease and are recommended to continue CMV monitoring after day 100 post-transplant
Number of Sites:	4
Study Duration:	4.5 years
Subject Participation Duration:	6-10 months
Description of Intervention:	CMV polymerase chain reaction (PCR) testing of a Dried Blood Spot (DBS) Sample
Objectives:	*Primary Objective:* - To evaluate adherence to recommended CMV monitoring duration and interval during the first year after transplant upon enrollment using subject self-collected dried blood spot testing *Secondary Objectives:* Evaluate the mean difference between the recommended monitoring that each subject completes between the DBS and the control arm Compare the incidence of CMV disease between the DBS monitoring and standard of care arms Evaluate the safety of DBS monitoring

Description of Study Design:	This is a randomized clinical trial to assess whether a subject centered, self-collection of DBS samples will improve compliance with the clinical recommendation of weekly CMV testing of HCT recipients who are at high risk for late CMV disease. Subjects will be randomized (2:1) to DBS monitoring or standard of care (per local institution) monitoring. Subjects in the DBS arm will receive training on the collection and shipment of the sample to a central laboratory for CMV PCR testing. Periodic whole blood samples also will be collected along with a DBS sample for plasma CMV PCR testing. Subjects in the standard monitoring arm will be followed per standard of care monitoring for their transplant center. Data from the CMV testing done at the local site will be collected from the subject's chart.
Estimated Time to Complete Enrollment:	18-24 months

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Despite strong evidence that preemptive therapy is highly effective in preventing cytomegalovirus (CMV) end organ disease after HCT, CMV monitoring adherence decreases significantly after day 100, when patients typically leave the care of the transplant team. Preliminary data (section 2.1.4.) indicate that fewer than 50% of patients who are recommended to continue CMV testing do so throughout the recommended testing period; the drop-off starts as soon as two months after discharge. Consequently, the incidence of late CMV end organ disease (defined as disease occurring after day 100 after HCT) remains high at 8% in the current era with recommended surveillance testing and preemptive therapy. Half these cases are CMV pneumonia, which continues to carry a substantial risk of death (section 2.1.3). In a recent multicenter randomized trial of prophylaxis compared to preemptive therapy for the prevention of late CMV disease the incidence of late CMV disease was only 2% [1]. During the trial, study coordinators were in close contact with study subjects, resulting in a CMV monitoring completion rate of 93%. Similarly, high rates of adherence are also routinely achieved during the first 3 months after HCT while patients are treated in specialized cancer centers, and these adherence rates correlate with CMV disease rates between 0 and 3%. However, outside of the clinical trial setting, the requirement for weekly blood draws is burdensome for patients late after HCT. In general, the frequency of CMV monitoring decreases as the frequency of doctor's office visits decreases, resulting in missed opportunities for preemptive therapy. Finger-stick collected DBS CMV testing would allow subjects to collect their samples at home and mail the cards directly to the laboratory. This method of CMV PCR testing has been validated in transplant recipients and has sufficient sensitivity to detect viral loads appropriate for a preemptive therapy approach late after transplantation [2,3].

In addition, mobile technology can be used to automate simple reminder and notification systems and to facilitate ongoing communication among the patient, their primary oncologist, and the transplant center. There is good evidence [4,5] that these types of reminders are efficacious for improving medication adherence; however, it has not yet been tested in the setting of post-transplant virologic monitoring.

2.1.1 CMV

CMV is a human herpesvirus known to infect 50-90% of US adults and is a major cause of morbidity and mortality in immunocompromised population. CMV infection can be acquired through multiple means, including: mother-to-child (in utero, breast milk), infected body fluids (saliva, genital secretions), blood transfusion, or organ transplant. The prevalence of CMV infection increases with age throughout life such that by age 90; ~90% of persons will have acquired CMV infection [6]. In immunocompetent persons, following primary infection by any of the routes noted above, CMV is controlled by the immune system and establishes latency ("dormancy") in multiple organs and cell-types for the life of the host. In particular, the lung represents one of the largest reservoirs of latent CMV in seropositive hosts, and may explain the propensity for CMV-associated pulmonary disease in predisposed hosts [7]. During periods of immunosuppression (or as a result of specific stimuli such as TNF-α, LPS, or catecholamines that are commonly associated with critical illness and sepsis [8]), CMV can reactivate from latency (preferentially in the lung) to produce active infection (viral replication). In persons with impaired cellular immunity, reactivation can progress to high-grade CMV replication, tissue injury, and clinically evident disease such as CMV pneumonia. Lower-grade CMV reactivation that is otherwise clinically silent ("subclinical") can also be detected in apparently immunocompetent persons with critical illness using sensitive techniques such as PCR [1]. In addition, even low-level, otherwise asymptomatic subclinical CMV reactivation can produce significant biologic effects both in vitro and in vivo, such as inflammation, fibrosis, and immunosuppression. These biological effects of CMV have been shown to occur through various mediators and other indirect means (reviewed in [9]). Importantly, several important CMV-associated adverse clinical outcomes in transplant populations (e.g. allograft rejection, secondary infections) are not necessarily accompanied by overt CMV disease and can only be detected by relatively sensitive means of virus detection such as PCR [10–12].

2.1.2 Importance and Risk Factors for Late CMV Disease after HCT

The phenomenon of late CMV disease was first recognized in the early 1990s when the first reports were published about effective prevention of early CMV disease, and, at the same time, cases of delayed onset CMV disease were observed [13]. Since then, a substantial body of literature indicates that CMV disease can be prevented during the first 3 months after HCT when antiviral agents are given prophylactically at engraftment or pre-emptively for pp65 antigenemia or detection of CMV DNA by PCR, with improved survival in selected high-risk patients [14,15]. However, once

prophylaxis is stopped or the preemptive strategy is applied less rigorously, CMV disease incidence rises again. As a result, the majority of CMV disease now occurs after day 100, resulting in a much longer period of high risk [2,3,14,16–19]. Late CMV disease (defined as disease presenting after day 100 after HCT) occurs primarily between day 100 and day 365 after transplant. Both late CMV infection and disease remain independent predictors for mortality after HCT, even though patients at highest risk for late CMV infection and disease can be identified by day 100 [2]. Without intervention, the incidence of late CMV disease ranges from about 10% to 17% of CMV seropositive recipients who are alive at day 100 [2]. Mechanistically, lymphopenia and delayed reconstitution of CMV-specific T-cell responses are the immunologic defects that predispose patients to late CMV complications [2]. In seronegative recipients with a positive donor (D+/R-), late CMV disease seems to occur almost exclusively in patients who had evidence of primary CMV infection during the first 100 days and the incidence of late CMV disease is similar to that observed in CMV seropositive recipients [3].

Approximately 50% of patients with late CMV disease present with pneumonia, followed by gastrointestinal disease (40%) and other manifestations such as retinitis, encephalitis, and late graft failure [3].

2.1.3 Outcome of Late CMV

The outcome of late disease remains poor, and late CMV disease is an independent risk factor for mortality in CMV seropositive recipients (adjusted hazard ratio 4.16 [95% confidence interval 2.7-6.5]) [3]. The outcome following pneumonia is worse than that following other manifestations of late CMV disease with 60% of patients dying within 6 months of late CMV pneumonia (Figure 1) [3,20]. In a recent multivariate analysis, the outcome of CMV pneumonia occurring in the late period was not different from that early after HCT [20].

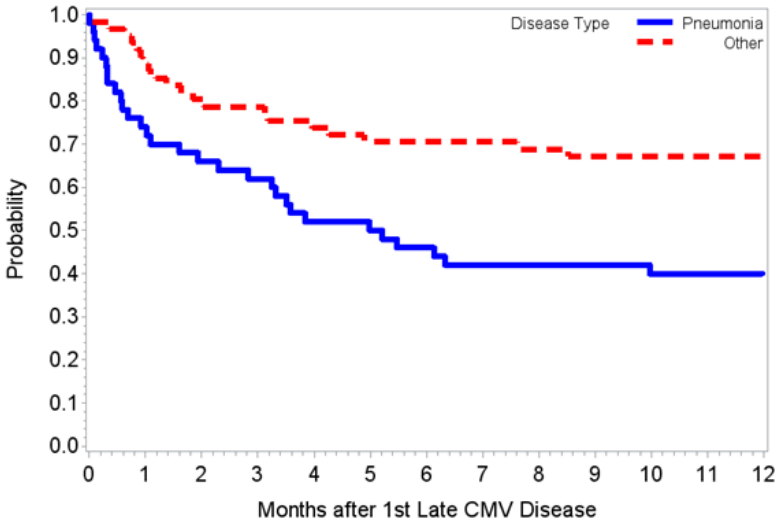


Figure 1. Kaplan-Meier plot of overall survival after late CMV disease, by disease type (n=117)

2.1.4 Approaches to Prevent Late CMV Disease

Although preemptive therapy based on virologic monitoring is the most commonly used strategy to prevent CMV disease during the first 3 months after HCT [21], maintaining regular weekly monitoring is often difficult late after HCT. Indeed, preliminary data demonstrate that outside a clinical trial setting (during which surveillance can be reinforced by study personnel), adherence to recommended surveillance recommendations is poor (Figure 2). This poor adherence is associated with continued high rates of late CMV disease.

There is compelling data from both cohort studies and multicenter clinical trials, that preemptive therapy strategies are highly effective when administered properly (Table 1). Moreover, a recent study showed excellent performance for PCR-based preemptive therapy (started at a plasma viral load ≥1000 IU/mL) [22]. In this study, the CMV disease incidence was similar to what has been accomplished in other recent multicenter randomized trials early after HCT (Table 1). This represents the first documentation in a randomized trial that preemptive therapy in the late setting can be highly effective if virologic monitoring is performed at >90% of expected time points. This rate contrasts starkly with what has been achieved with patients outside the clinical trial setting and provides the rationale for the key hypothesis of this proposal that home-based, mobile technology supported virologic monitoring will

result in adherence rates associated with effective preemptive treatment strategies (see Table 1).

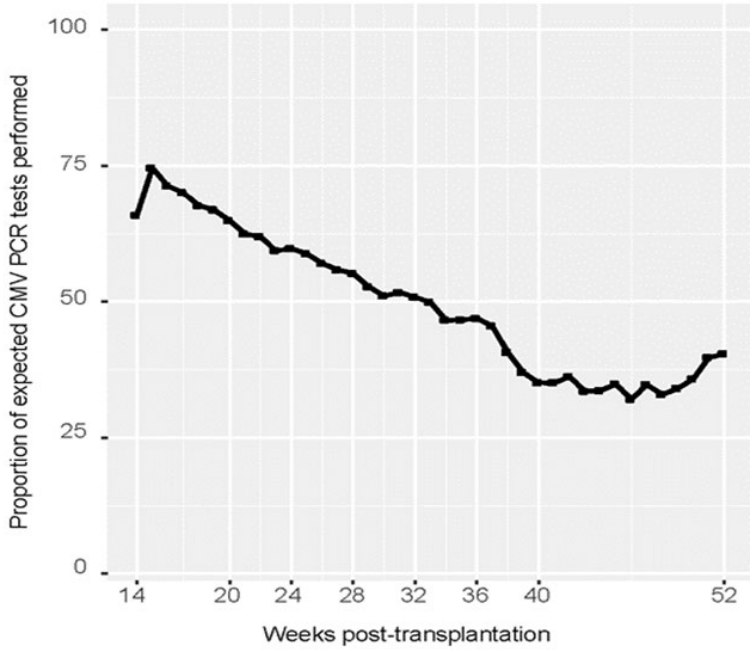


Figure 2. Proportion of expected CMV monitoring tests performed by week after day 100 (14 weeks) post-transplant in patients for whom extended monitoring is recommended (review of 1246 HCT recipients at risk of CMV disease)

Table 1. Effectiveness of preemptive therapy strategies in recent randomized trials with high viral monitoring adherence (placebo group) [22–25]

Author	Journal	Year	N	Period	CMV Disease Incidence
Marty et al.	Lancet ID	2011	227	Early	2.4%
Marty et al.	NEJM	2014	59	Early	3.0%
Chemaly et al.	NEJM	2014	33	Early	0%
Boeckh et al.	Ann Int Med	2014	89	Late	2.0%

Several novel CMV prevention strategies are or have recently been under investigation, including new drugs (e.g., letermovir, brincidofovir, and maribavir) and CMV vaccines [23–28]. It is unclear whether the drugs will be efficacious or will be suitable for long-term use due to unknown long-term toxicities and cost. In addition, even if effective and non-toxic new anti CMV drugs become available, long term use of these therapies may delay CMV-specific immune reconstitution or promote resistance [29,30], or, in the case of the vaccine, may be less effective in the most

immunosuppressed patients [28]. Thus, for years to come viral monitoring will likely be an essential part of preemptive therapy strategies.

2.1.5 Dried Blood Spot CMV Testing

DBS testing has been increasingly utilized to detect CMV in studies of congenital CMV by using stored Guthrie cards [31–33]. Self-collection of samples using swabs to examine the biology of herpesviruses (including CMV) is well established in healthy volunteers and HIV-infected subjects. It is not known whether giving subjects the ability to self-collect samples will improve compliance or whether CMV monitoring via DBS testing is sufficiently sensitive to detect re-emergence of late occurring CMV viremia.

2.1.5.1 Dried Blood Spot Assay Design History

The DBS PCR assay was initially developed in 2001 using two primer/probe pairs, one of which amplifies the gB region and one the IE region of the CMV genome. Over the last 10 years, this PCR assay has been extensively used in clinical research to study CMV infections of immunocompromised patients at both the University of Washington (UW) and the Fred Hutchinson Cancer Research Center (Fred Hutch) and has proven to be a valuable tool for detection and monitoring of CMV reactivation and clinical disease.

2.1.5.2 Summary of DBS Assay Performance vs Standard Plasma PCR Clinical Assay

The performance of the current DBS PCR assay was compared to an ultrasensitive plasma PCR assay (lower level of quantitation 50 IU/mL) that is used clinically at the University of Washington Clinical Virology Laboratory to monitor patients for CMV infection. The standard used in the clinical PCR assay is the WHO International Standard for human cytomegalovirus (HCMV). Residual whole blood samples from specimens that were submitted for clinical plasma PCR testing were also examined by the DBS assay. The primary goal of the study was to determine whether the DBS assay detected CMV DNA in whole blood samples that were determined to be positive at the 1000 (3.0 log 10) IU/mL level in the clinical PCR assay, a level that is commonly used to trigger preemptive treatment of patients in the late period after hematopoietic cell transplantation (HCT). As a secondary goal, the study also examined the ability of the DBS to detect CMV DNA in whole blood samples that are positive at the 500 (3.0 log10) IU/mL level, a level also used by some sites to start preemptive therapy.

Residual samples from patients who had plasma viral loads ≥150 (2.18 $\log_{10}$) IU/mL were spotted on to filter cards (50uL). The residual blood sample was used with 24-72

hours after the sample had been drawn. A total of 145 clinical samples from 69 persons were collected and tested. From the 145 clinical samples, 22 had plasma CMV viral load ≥1000 IU/mL; 48 had known plasma CMV viral load ≥500 IU/mL and 97 had viral loads ≤ 500 IU/mL . The results of the comparisons at two different thresholds are summarized in the table below.

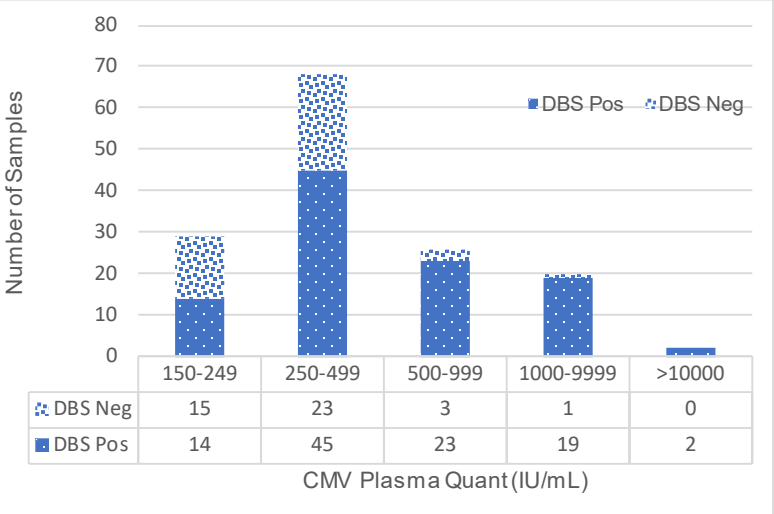
Table 2. Summary of DBS assay evaluation

Clinical plasma PCR and DBS PCR results with different thresholds	**N /Total**
Clinical Plasma CMV PCR ≥500 (2.7 $\log_{10}$) IU/mL	48/145
Clinical plasma CMV PCR ≥500 IU/mL (2.7 $\log_{10}$) **and** DBS positive	44/145
(Clinical PCR range: 510-36000 [2.7-4.6 $\log_{10}$] IU/mL)	
Clinical plasma CMV PCR <500 IU/mL (2.7 $\log_{10}$) and ≥150 (2.18 $\log_{10}$) IU/mL	97/145
Clinical plasma CMV PCR <500 IU/mL (2.7 $\log_{10}$) and DBS positive	59/145
(Clinical PCR range: 41-490 [1.6-2.7 $\log_{10}$] IU/mL)	
Clinical plasma CMV PCR ≥1000 (3.0 $\log_{10}$) IU/mL	22/145
Clinical plasma CMV PCR ≥1000 (2.7 $\log_{10}$) IU/mL and DBS positive	21/145
(Clinical PCR range: 1000-36000 [3.0-4.6 $\log_{10}$] IU/mL)	
Clinical plasma CMV PCR <1000 (3.0 $\log_{10}$) IU/mL and ≥150 (2.18 $\log_{10}$) IU/mL	123/145
Clinical plasma CMV PCR <1000 (2.7 $\log_{10}$) IU/mL and DBS positive	82/145
(Clinical PCR range: 41-970 [1.6-2.9 $\log_{10}$] IU/mL)	

Samples with a viral load ≥1000 (2.7 $\log_{10}$) IU/mL were positive on the DBS assay 95% of the time (21/22 samples). Samples with viral loads between 150 (2.18 $\log_{10}$) IU/mL and 1000 (2.7 $\log_{10}$) IU/mL were positive at a lower rate (67%).

The proportion of DBS CMV PCR positive samples relative to plasma PCR positive samples in different plasma viral load ranges is visually depicted in Figure 3.

Figure 3. Distribution of DBS CMV PCR result based on plasma CMV viral load across different viral load ranges (columns).



2.1.5.3 Limit of Detection

Based on a complementary log-log model, the rate of detection or sensitivity of the DBS assay at 2.99 $\log_{10}$ IU/mL CMV in plasma is above 95% (Figure 4. Thus, the limit of detection would be about 2.98 logs or $10^{2.98}$ = 955 IU/ml (see the reference lines at x-axis of 2.99 and y-axis of 0.95). Similar results are obtained compared to these only the first sample date was used per person (69 samples), with a cut off of 2.91 on the $\log_{10}$ scale or 812 IU/mL. Based on this model, the sensitivity at 500 (2.70 $\log_{10}$) IU/mL is 85%, and the sensitivity at 1000 (3.00 $\log^{10}$) IU/mL is 96%.

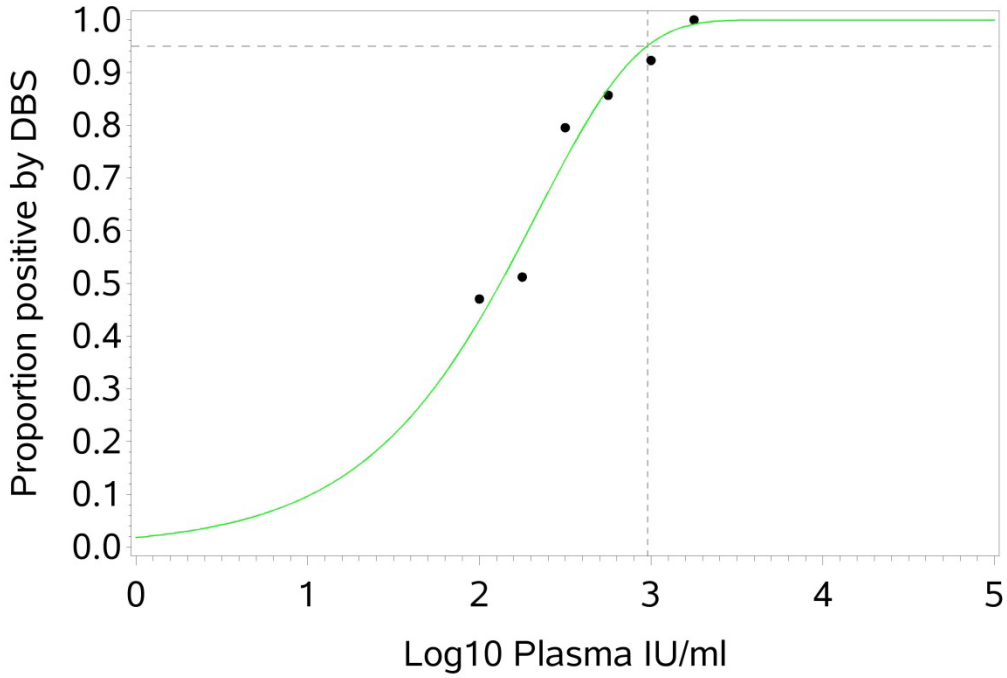


Figure 4. Complementary log-log model to compare CMV viral load in plasma and the sensitivity of DBS

Overall, the results of the most recent study shown above as well as prior clinical results using earlier versions of the assay suggest that the DBS assay reliably detects viral loads of ≥1000 (3.0 $\log_{10}$) IU/mL highly reliably and that the sensitivity is somewhat lower between 500 (2.7 $\log_{10}$) and 1000 (3.0 $\log_{10}$) IU/mL.

2.1.6 Mobile Communication

The use of mobile technology to increase adherence to medical therapies has increased dramatically in recent years [5]. In this study, mobile technology will be used

to remind HCT survivors to perform CMV monitoring using finger-stick collected DBS testing in their home setting or to visit their doctor's office to perform the test.

2.2 Rationale

Given accumulating evidence that preemptive therapy is effective against CMV disease in the HCT setting with high (i.e., 90%+) adherence to the recommended CMV testing schedule [22,24,25,35], a critical question is whether innovative easy-to-perform self-testing assisted by mobile technology reminders can improve monitoring frequency to the high rates needed in the late setting without the hands-on support that is available in the early post-transplant setting. This is a proof-of-concept, multi-site, randomized clinical trial to test whether subject-collected dried blood spot samples for CMV PCR testing can improve adherence to recommended CMV monitoring late after transplant compared to the standard of care. The study will enroll HCT recipients following their discharge from their transplant center who are, therefore, at risk for late CMV disease. Assessing adherence in this population to the recommended CMV testing schedule is critical to evaluating whether adopting DBS self-collection can improve the efficacy of preemptive therapy strategies and, therefore, potentially reduce the morbidity and mortality associated with late CMV disease.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

There are few potential risks associated with this study. Risk is primarily derived from the needle stick. The following table presents risks based on experience with self-administered finger stick procedures. In theory, there is a risk of missing CMV cases due to the sensitivity limit of the DBS tests. This information will be communicated to subjects in the sample informed consent form.

Table 3. Summary of potential risks

Less common	Pain and/or bruising at puncture site
Uncommon or rare	Missed CMV cases due to sensitivity limits of DBS test
Uncommon or rare	Infection at puncture site

2.3.2 Known Potential Benefits

Study subjects may benefit from more frequent CMV monitoring and, as a result, more timely and effective initiation of preemptive therapy for CMV disease. It is also possible, though, that a study subject may not derive any benefit from participation in this trial.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Hypothesis

Home-based, mobile technology supported virologic monitoring will result in adherence rates associated with effective preemptive strategies against late CMV end organ disease

3.1.2 Primary Objective

To evaluate adherence to recommended CMV monitoring duration and interval during the first year after HCT upon enrollment using subject collected dried blood spot testing

3.1.3 Secondary Objectives

1. Evaluate the mean difference between the recommended monitoring that each subject completes between the DBS and the control arm
2. Compare the incidence of CMV disease between the DBS monitoring and standard of care arms
3. Evaluate the safety of DBS monitoring

3.1.4 Exploratory Objectives

1. Evaluate the transit time from self-collection to arrival in the laboratory
2. Assess the mechanism for non-compliance as defined by a missing DBS sample PCR result (e.g. mobile technology failure, sample collection failure, sample delivery failure, sample viability failure)
3. Compare the performance characteristics of concurrently drawn DBS with plasma CMV PCR (e.g., sensitivity and specificity concordance)
4. Determine if the randomized study population is representative of the population as a whole
5. Obtain a population-based estimate of late CMV disease in observational and randomized cohorts
6. Describe local provider CMV treatment algorithms
7. Assess subject and provider satisfaction of DBS testing

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

The number of participants who have completed >90% of their recommended CMV monitoring tests at one year after HCT in the DBS and control arms

3.2.2 Secondary Outcome Measures

1. The total number of recommended CMV monitoring tests that were completed per subject by 1 year after HCT
2. Number of subjects in DBS and standard of care arms with end-organ CMV disease, possible and proven/probable[36] by 1 year after HCT (Appendix and MOP); CMV syndrome will not be used to define CMV disease
3. Number of subjects with finger-stick procedure-related Grade 3 AEs at 1 year after HCT

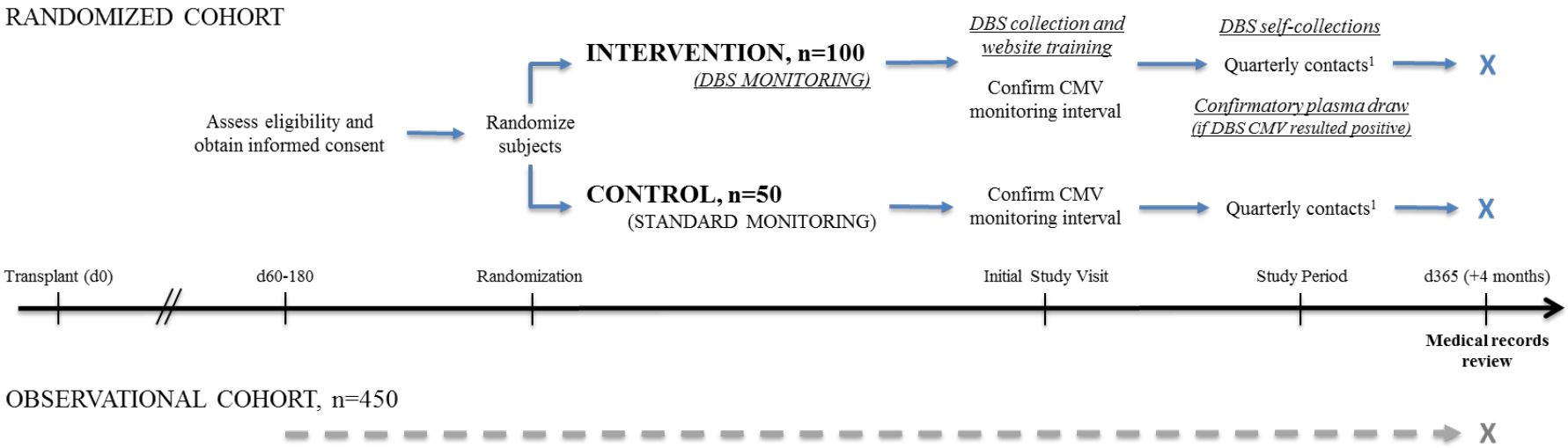
3.2.3 Exploratory Outcome Measures

1. Mean time from scheduled pick-up at subject residence (DBS tests) or blood draw facility (plasma sample) to arrival at the laboratory (hours) at all applicable test points throughout the study period
2. Number of mobile technology failures, sample collection failures, sample delivery failures, and sample viability failures.
3. Sensitivity, specificity and predictive values of CMV detection in DBS versus plasma testing
4. Number and type of key transplant characteristics in randomized subjects and observational subjects (Section 5.1)
5. Describe the baseline patient demographics of randomized cohort and observational cohort.
6. Number of participants with end-organ CMV disease (possible and proven/possible) at 1 year after HCT in randomized subjects and observational subjects
7. Describe local provider CMV treatment viral load treatment thresholds
8. Describe local provider CMV monitoring interval recommendations
9. Report composite scores of different variables of subject and provider satisfactions according to the 5-point Likert scale questionnaires.

4 STUDY DESIGN

This is a randomized clinical trial to assess whether self-collection of samples (DBS) that are mailed for testing to a central laboratory will improve the compliance with the clinical recommendation of weekly CMV testing of HCT recipients. 150 allogeneic HCT recipients ≥15 years of age, who are considered by their transplant teams to be at risk for late CMV disease and who are clinically recommended to continue CMV monitoring after day 100 post-transplant, will be randomized (2:1) to subject self-collected DBS CMV monitoring with mobile technology support or standard of care with office-based testing. Randomization will occur when discharge from the transplant service is imminent, generally near day 100 post-transplant (enrollment window day 60-180 post-transplant). DBS self-collections will start the week after discharge. More than 85% of late CMV disease occurs within the first year after HCT [37]. While most patients are discharged to long-term primary care providers approximately 100 days after HCT, some may be discharged earlier, and some may stay longer with the transplant team due to complications. These latter patients are often at particularly high risk for late CMV complications and thus are important candidates for participation in this trial. For this reason, study entry is allowed up to 180 days (~6 months) after HCT and the duration of study participation is anticipated to be within a range of 26 weeks to 43 weeks.

The transplant centers participating in this study have either a standard process where transplant recipients are offered to sign a consent to allow data from their charts to be accessed for retrospective trials or a waiver of additional consents for accessing charts for retrospective trials. This offers an opportunity to assess whether the study sample is representative of HCT population as a whole and to obtain a population-based estimate of later CMV disease. Therefore, clinical charts from an additional 450 HCT recipients (Observational Cohort) who meet eligibility criteria and have already consented for retrospective studies at the enrolling sites but are not participating in the DBS testing for CMV will be reviewed for the incidence and timing of CMV disease, morphologic relapse of the underlying disease, and death. Data from these subjects will be used to assess whether the randomized study sample is representative of the DBS study population and to obtain a population-based estimate of late CMV disease.



[1] Quarterly contacts will be scheduled from the date of transplant, not based on the date of enrollment.

Figure 5. Schematic of Study Design

4.1 Randomized Cohort

Following consent, subjects in the DBS arm of the study will be asked to perform weekly DBS collection starting the week after discharge, once appropriate training has been provided (collection can be performed by a caregiver as long as appropriate training has been completed). Weekly reminders will be sent either as email or text messages. DBS samples will be mailed directly to the central lab (University of Washington) via pre-paid overnight mail (pick-up can be scheduled via the website or by phone through website) by the subjects. Results will be transmitted via a secure server to participating site staffs. The research staff will then contact the subjects' treating physicians via phone/fax notifying of positive results. The subjects will receive a notification via the study web portal when their positive CMV test results are available with a link to the secure website that can be used to access them. Text message reminders have been effective in randomized trials of medication adherence [5,38,39], but too frequent reminders may be counterproductive. The web/mobile technology employed in this trial allows subjects to select method of electronic reminders (email and/or text message) and day and time of weekly electronic reminders. At minimum, one reminder will be sent before the recommended test.

In addition to the DBS Self-Collection Kits, subjects will be provided with Confirmatory Whole Blood Collection Kits for a plasma CMV PCR test (one 6 ml EDTA tube and a DBS self-collection kit with shipping material). When subjects are notified of a positive DBS result (a reminder will be provided with the notification of positive test results) they will take the Confirmatory Whole Blood Collection kit to their clinical blood draw facility and get their samples collected as soon as possible. The clinical laboratory staff will draw the additional blood and use the pre-paid FedEx shipping kit to send the sample to the University of Washington Molecular Virology Laboratory for testing. Decisions whether to administer preemptive treatment based on plasma viral load results will be made by the primary care provider of the subject. At the time of office-based blood draw for research confirmatory plasma CMV PCR, the subject will perform an additional DBS test, which will be sent together with the plasma sample to the central lab. These samples will provide additional virologic data to assess performance of the DBS assay. This method has been used successfully in a recently completed phase III trial [22].

Training of participants on the DBS self-collection procedures will include three training sessions with a study coordinator. The first session will describe, observe, and provide guidance on DBS self-collection procedures and web/mobile technology. The second session will be a supervised session with the subject to observe and answer questions. It is preferable that supervised DBS trainings with a study coordinator are done in-person; however, if this is

not possible these supervised trainings may be completed remotely via video conferencing as an option (See MOP for details and instructions). The third session will be unsupervised with the subject performing the testing in their home or temporary residence nearby the transplant center This training session will be followed up with a phone call or in-person check-in and a questionnaire to assess understanding. At each session, the subject will have written instructions on performing the DBS testing and utilizing the web/mobile technology available for their reference. Each DBS training session requires the completion of a DBS collection. The study coordinator will be available to provide additional training session as needed. In addition, DBS collection instructional video is available on the study website. Prior to discharge from the transplant care team, study personnel must confirm that the subject demonstrates proficiency in DBS sample collection and use of the study website.

The mobile technology will consist of a notification system that sends weekly reminders by email and/or text message determined by the subject's preference to the subject to complete DBS self-collection procedures. The notifications are sent from a web-based, 21 CFR 11-compliant electronic data capture system created and maintained by The Emmes Company. The subject will log into the system on a computer or mobile device with internet access to set up study reminders and record sample collection and shipment information. The system will also house IRB-approved training materials and the web address of the United States Postal Services (USPS) so that shipments may be scheduled.

Subjects who are enrolled in the control arm of the randomized study will continue to receive their office-based CMV plasma testing as recommended by standard of care.

Quarterly follow-up contacts will be made with the primary care physicians of all randomized subjects enrolled in the study to obtain each subject's medical record. Data from each subject's medical record on immunosuppression, CMV disease status, any interim use of antiviral therapy for CMV disease or plasma reactivation, clinically recommended CMV test interval, any hospitalization record, relapse, and survival status will be collected.

4.2 Observational Cohort

A parallel observational contemporary cohort will be established of up to 450 transplant recipients who are eligible for enrollment but declined participation as part of the randomized cohort and/or have consented at their site to have data from their charts abstracted for retrospective studies and meet entry criteria for the randomized trial. Selection of eligible subject charts will be based on similar criteria to the randomized study and will include subjects who qualified for the study but did not provide consent. Eligible participants meeting those criteria will be included in an observational study.

To allow data extraction for this observational cohort, each participating site must have on record with their IRBs either:

- A general consent for the review of medical records for retrospective studies that can be utilized for this activity, or

A waiver of additional consent for the review of medical records for retrospective studies linked to this protocol. Data from each subject's medical record on immunosuppression, steroid use, CMV disease status, any interim use of antiviral therapy for CMV disease or plasma reactivation, clinically recommended CMV test interval at time of discharge, morphological relapse, and survival status will be collected at approximately one year post transplant date. Data to be abstracted from the charts will cover an equivalent length of time as those subjects enrolled in the randomized study (i.e. until 1 year after HCT).

5 STUDY ENROLLMENT AND WITHDRAWAL

Approximately, 150 allogeneic HCT recipients ≥15 years of age, who are considered by their transplant team to be at risk for late CMV disease as defined in the entry inclusion criteria and recommended to continue CMV monitoring after 100 days post-transplant, will be enrolled in the study. These individuals will be randomized (2:1) to subject collected DBS CMV monitoring with mobile technology support or standard care with office-based testing.

Recruitment of subjects will follow local recruitment and enrollment policies and procedures. The study sites will request a waiver of informed consent and a waiver of HIPAA Authorization for pre-screening purposes (to determine CMV sero- and reactivation status) from their local Institutional Review Board (IRB). Upon approval of both waivers, research staff will review the electronic medical records to pre-screen subjects who will undergo or have undergone an allogeneic hematopoietic cell transplant (HCT). Pre-screening will be done by trained and experienced research staff who will review the electronic charts using a standardized screening tool. In addition, potential subjects may be identified by the attending physician (or team) based on the eligibility criteria approximately one month prior to anticipated discharge from the primary transplant team. If a subject is identified as meeting the inclusion/exclusion criteria, the PI or designee will explain the study and will seek informed consent to study participation.

Retention efforts will focus on the interval between enrollment/randomization and discharge from the transplant team and start of diagnostic monitoring. Since adherence is a key study endpoint, direct contact between site personnel and study subjects will be kept as equal as possible between the two arms to avoid biasing the comparison of CMV monitoring adherence.

5.1 Subject Inclusion Criteria

5.1.1 Randomized Cohort

- Must be ≥15 years of age at the time of enrollment
- Must be able to provide written consent and complete the informed consent
- Must have received allogeneic hematopoietic cell transplantation within 60-180 days prior to randomization
- CMV seropositive or had a donor who was CMV positive

- One or both of the following:
    - CMV event[1] within the first 100 days post-transplant requiring anti-viral treatment
    - Receipt of CMV prophylaxis[2] (for at least 30 days) prior to randomization. Continuation of letermovir or acyclovir/valacyclovir (high and low dose) prophylaxis after day 100 per institutional standard of care is permitted.

[1] *CMV event defined as DNA detection or disease*

[2]*Anti-viral treatment or prophylaxis includes ganciclovir, valganciclovir, foscarnet, letermovir, maribavir or acyclovir/valacyclovir (high and low dose)*

- Direct availability to the internet either by a computer in the residence or a smart phone
- Had at least one or more of these conditions:
    - HLA mismatch[3]
    - Umbilical cord blood source[4]
    - GVHD[5]
    - T-cell depletion[6]

[3]*Human leukocyte antigen (HLA)-related(sibling) donor with at least one mismatch at one of the following three HLA-gene loci: HLA-A, -B or -DR, Haploidentical donor, Unrelated donor with at least one mismatch at one of the following four HLA-gene loci: HLA-A, -B, -C and -DRB1*

[4]*Use of umbilical cord blood as stem cell source*

[5]*Acute or chronic GVHD requiring topical steroid for GI GVHD and/or systemic steroid treatment (≥1mg/kg/day of prednisone or equivalent dose of another corticosteroid) within 6 weeks prior to enrollment*

[6]*Subjects who have received partial or full T-cell depletion (with or without GVHD). T-cell depletion can be given as either ex-vivo or in-vivo for GVHD prophylaxis. T-cell depleting agents include, but are not limited to, anti-thymocyte globulin (ATG) and alemtuzumab.*

5.1.2 Observational Cohort

- Must be ≥15 years of age at the time of enrollment
- Must have one of the following:
  - Consented for retrospective studies at their transplant center, or
  - Be included under the auspices of the site's IRB approved waiver of additional consent for retrospective studies
- Must have received allogeneic hematopoietic cell transplantation during or within 1 year prior to the conduct of the randomized trial (defined as time during which randomization is done).
- CMV seropositive or had a donor who was CMV positive
- One or both of the following:
  - CMV event[1] within the first 100 days post-transplant requiring anti-viral treatment.
    - Receipt of CMV prophylaxis[2] (for at least 30 days) prior to registration. Continuation of letermovir prophylaxis or acyclovir/valacyclovir (high and low dose) after day 100 per institutional standard of care is permitted.

  [1] *CMV event defined as DNA detection or disease*

  [2]*Anti-viral treatment or prophylaxis includes ganciclovir, valganciclovir, foscarnet, letermovir, maribavir or acyclovir/valacyclovir (high and low dose)*
- Meet one or more criteria of the following:
  - HLA mismatch[3]
  - Umbilical cord blood source[4]
  - GVHD[5]

- T-cell depletion[6]

[3]Human leukocyte antigen (HLA)-related(sibling) donor with at least one mismatch at one of the following three HLA-gene loci: HLA-A, -B or -DR, Haploidentical donor, Unrelated donor with at least one mismatch at one of the following four HLA-gene loci: HLA-A, -B, -C and -DRB1

[4]Use of umbilical cord blood as stem cell source

[5] Acute or chronic GVHD requiring topical steroid for GI GVHD and/or systemic steroid treatment (≥1mg/kg/day of prednisone or equivalent dose of another corticosteroid) within 6 weeks prior to enrollment

[6] Subjects who have received partial or full T-cell depletion (with or without GVHD). T-cell depletion can be given as either ex-vivo or in-vivo for GVHD prophylaxis. T-cell depleting agents include, but are not limited to, anti-thymocyte globulin (ATG) and alemtuzumab.

5.2 Subject Exclusion Criteria

5.2.1 Randomized Study

- Inability to fully comprehend the study website and study procedures
- Any other condition, which in the opinion of the investigator would interfere with successful completion of this clinical trial
- Morphological relapse (bone marrow or peripheral blood blast) prior to registration.

5.2.2 Observational Cohort

- Did not meet all inclusion criteria
- Morphological relapse (bone marrow or peripheral blood blast) prior to registration.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Subjects will be randomized in a 2:1 ratio to subject-collected DBS CMV monitoring with mobile technology support (n=100) or standard care with office-based testing (n=50). Additional subjects will be enrolled as retrospective observational controls and will not be randomized (n=450). The list of randomized treatment assignments will be prepared by statisticians at The Emmes Corporation (Emmes) and included in the enrollment module of Emmes' Internet Data Entry System (IDES). IDES will assign each subject a study arm from the list after demographic (date of birth, race/ethnicity, and sex) and eligibility data have been entered into the system. Randomization will be stratified by transplant site and subject's perceived ease of access to blood draw facility. Observational cohort will be enrolled in IDES after the required demographic and eligibility information are entered. Instructions for use of the enrollment module are included in the IDES User's Guide. Manual back-up randomization procedures are provided in the MOP for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

Subject IDs will be assigned to potential subjects, who have documented informed consent by signing an Informed Consent form, as they are screened. The subject ID will be used for all communications with outside institutions to ensure confidentiality.

Subjects who are randomized and drop out of the study prior to beginning follow-up (first scheduled test both arms) will be replaced. Over-enrollment is allowed to accommodate competitive enrollment and accommodate replacement subjects.

5.3.2 Masking Procedures

None

5.3.3 Reasons for Withdrawal

Any enrolled subject may withdraw or be withdrawn from the study for the following reasons:

- The subject (guardian) withdraws consent
- The study is terminated
- Subject fails to demonstrate proficiency in DBS sample collection

- For any reasons that, in the opinion of the investigator, precludes the subject's participation in the study
- Subject has been enrolled inappropriately based on inclusion/exclusion criteria; these subjects may be replaced, or
- Initiation of end of life care
- If the subject has morphological relapse prior to the start of study related CMV surveillance testing.

Subjects may withdraw from participation in the study at any time. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs), although it is recognized that the subject can refuse to provide a reason. If a randomized subject is withdrawn prior to the first scheduled study test (or prior to the start of late CMV surveillance) for any reason, they will be replaced; however, they will not be moved over to the observational cohort.

For subjects who withdraw or are withdrawn prior to completion of the study, clinical information relevant to the secondary endpoint and the SAEs of special interest (proven/probable CMV disease) should continue to be obtained from the primary physicians' office or medical records until the end of the follow-up unless the subject withdraws consent .

The NIAID/DMID, the IRB and/or the FDA have the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to the following:

- Incidence of AE's indicating a potential health hazard
- Data recording is inaccurate or incomplete
- The Investigators has not been adhering to the protocol or applicable regulatory guidelines in conducting the study.

5.3.4 Handling of Withdrawals

Eligible subjects who withdraw from the study between randomization and the first scheduled study test (or prior to the start of late CMV surveillance) may be replaced. Reasons for withdrawal will be recorded in the Consort diagram.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

6.1.1 DBS Self-Collection Kit

The HTL Strefa Acti-Lance Safety Lancet (Universal) has been selected as the finger stick device for this trial. This lancet has a quick incision (0.002 seconds) and the 23-gauge needle penetrates 1.8mm, minimizing pain and bruising to the subject. This lancet has a closed design, which ensures that the needle remains inside the lancet before and after the puncture. DBS Self-Collection Kit will be assembled, packaged and distributed to each enrolling site by Fred Hutch (Table 4); the kit will be stored and shipped at room temperature. Each clinical site is responsible for supplying enrolled subjects with collection kits, which includes detailed instruction of use (refer to MOP). Biohazard sharps container will be provided with the initial supply to the subjects for disposal of lancets. Refer to MOP for detailed and updated instructions on study kits and supplies.

Table 4. Contents of DBS Self-Collection Kit

Item	Quantity
Lancet	1
Sterile Alcohol Prep Pad	1
Gauze Pad	1
Bandage	1
Whatman 903 Elute Card	1
Subject Label	1
Whatman FTA Multi-Barrier Pouch	1
Desiccant Pack	1
Biohazard Specimen Bag	1
Padded Envelope with Return Address Label	1
DBS Self-Collection Kit Instruction of Use	1

6.1.2 CMV PCR

The DBS CMV assay consists of two major steps, an extraction to elute DNA from Whatman 903 Protein Saver Cards and purify it from other blood components on the DBS, followed by a real-time PCR to quantify the CMV present. The CMV PCR assay was initially developed in 2001 using two primer/probe pairs, one of which amplifies the gB region and one the IE region of the CMV genome. Over the last 10 years this PCR assay has been extensively used both clinically and to study CMV infections of immunocompromised patients at both at the University of Washington and the Fred

Hutch. This assay has proven to be a valuable tool for detection and monitoring of CMV reactivation and clinical disease.

The DBS testing has been increasingly utilized to detect CMV in studies of congenital CMV by using stored Guthrie cards [31–33]. A critical element, which has a significant impact on the assay sensitivity, is the extraction method. A variety of extraction methods have been evaluated in the CAP/CLIA certified University of Washington Molecular Virology Laboratory to identify the method that gives maximum recovery of the virus from the filter paper but elutes the minimum amount of PCR-inhibiting polysaccharides. A study showed good correlation between the levels of CMV in plasma and DBS samples of 35 solid organ transplant patients, using a validated method of Whatman 903 Protein Saver Cards and a chemical extraction with Chelex developed at the University of Washington Molecular Virology Laboratory. The extraction method for the current DBS CMV Testing Device has been modified to use the extraction kit available on the Promega M16 MDx instrument, because of its ability to automate the extraction and to obtain an increased CMV yield.

6.2 Assessment of Subject Compliance with Study Intervention/Investigational Product

Subject compliance will be measured by questionnaires as described in section 7.4.

6.3 Concomitant Medications/Treatments

6.3.1 Randomization

Any of the following medications, if applicable, that were administered within 14 days prior or ongoing at the time of randomization will be captured and reported to Advantage EDC[SM]:

- Steroids, topical only for GI GVHD and systemic (within 6 weeks prior to randomization)
- PUVA
- Immunosuppressant for acute or chronic GVHD (within 14 days prior to randomization, collect only start and stop dates)
- Preemptive or prophylactic antivirals (i.e. dosing of foscarnet, ganciclovir, valganciclovir, cidofovir, brincidofovir, letermovir, acyclovir/valacyclovir [high

and low dose], or any other investigational anti-CMV agent) (within 14 days prior to randomization).

- T-cell depleting agents including, but not limited to, anti-thymocyte globulin (ATG), alemtuzumab, etc. (any time after conditioning)

6.3.2 Quarterly Follow-Up

Following medications, if applicable, will be captured and reported to Advantage EDC^{SM} during medical chart review at quarterly follow-up:

- Antiviral used for CMV treatment
- Steroids, topical only for GI GVHD and systemic

7 STUDY SCHEDULE

7.1 Screening

Every inclusion and exclusion criteria will be assessed by reviewing medical records or databases by the study coordinators. These will then be confirmed by site principal investigators to proceed for an informed consent meeting.

7.2 Enrollment/Baseline and Initial Study Visit

Enrollment will occur when discharge from the transplant service is imminent, generally near day 100 post-transplant (enrollment window day 60-180 post-transplant). The schedule of procedures is summarized in Appendix C.

At the enrollment visit, the following procedures will be performed for all study participants (e.g. DBS, Standard of Care, and Observational cohorts):

- Confirm eligibility (if screening and enrollment is not done on the same day)
- Confirm or obtain consent prior to proceeding with any study activities. (Refer to Section 4.2 for Observational cohort enrollment)
- Confirm recommended monitoring schedule.
- Randomization (not applicable for observational cohort)
- Counseling on the importance of adherence to the recommended CMV monitoring schedule (not applicable for Observational cohort)
- Collection of baseline data from subject charts (see manual of procedures)
    - Age
    - Demographics
    - Medical history relating to:
        - Transplant
        - GVHD
        - CMV plasma reactivation or disease
    - Concomitant Medications

7.2.1 Enrollment and Initial Study Visit Procedures for Randomized DBS Arm

- Provision of DBS Self-Collection Kits (Refer to Section 6.1.1 and MOP) at each enrolling site (kits are assembled and distributed to each enrolling site by Fred Hutch)
- Training in finger-stick DBS collection for subjects and/or caregivers and sample shipping (refer to MOP)
- Training on the use of web and mobile technology tools with instructions provided in the manual of procedures from Emmes.
  Training includes:
  - Creation of login ID and password for website
  - Setting preferred communication method (SMS text or email)
  - Setting timeframe for receiving reminders and notifications
  - Using study website and mobile technology component
- Provision of Confirmatory Whole Blood Collection Kit (each kit to include one 6 mL EDTA tube with shipping box) at each enrolling site (kits are distributed to each enrolling site by Fred Hutch)
- Confirmation of integrity of the initial supply of DBS and plasma kits
- Instruction to provide plasma for central lab PCR testing at clinical blood draws

7.2.2 Enrollment Procedures for Randomized Standard of Care (SOC) Arm

Subjects who meet all inclusion and exclusion criteria, signed DBS CMV protocol consent form to participate in the trial, and randomized into standard of care arm will provide the same baseline characteristics via review of medical records. All following study activities of SOC arm, other than DBS collection, will be the same as DBS arm.

7.2.3 Enrollment Procedures for Observational Cohort

Subjects who meet all inclusion and exclusion criteria, declined DBS CMV protocol, but have signed medical records review consent at each participating transplant center will be enrolled in the control arm for retrospective chart review for CMV testing frequency at the time of discharge from transplant clinic, CMV disease, relapse, and mortality up to 1 year after HCT. Refer to Section 4.2.

7.3 Weekly DBS Testing

Subjects randomized to the DBS arm are instructed to collect DBS on the same day and at approximately the same time each week (Sunday through Wednesday to avoid delays due to weekend shipping) following the provided instructions (refer to MOP). Subjects will start weekly

DBS collections the week after discharge and continue until approximately 1 year after HCT even following a positive result or initiation of preemptive therapy. If a DBS participant is hospitalized, the participant should not collect weekly DBS samples during hospitalization. The admitting hospital should monitor the DBS participant for CMV with at least weekly plasma CMV DNA PCR testing during hospitalization. Once discharged, the study team will remind the participants to resume DBS collections according to their weekly schedules. The study team may provide a refresher DBS training if needed. If a subject's DBS result is positive, and/or prior to any initiation of standard-of-care preemptive therapy, a 6ml whole blood (EDTA anti-coagulant tube) will be collected for plasma CMV PCR analysis by the subject's physician or referred collection site and an additional DBS sample at their scheduled laboratory visit with exemption to emergent hospital visit or hospitalization. Subjects will be provided with a pre-paid mailer with instructions to deliver to the collection center to assist with plasma CMV sample shipment to University of Washington Clinical Virology Laboratory on the day of collection. The subject will need to collect a DBS test at the time of the plasma collection. Subjects will be provided with a DBS kit to take with them to the physician's office. USPS should be contacted from the physician's office for pickup of the completed DBS kit.

All test results will be transmitted via a secure server to the study sites from the laboratory. The research staff will then contact the subjects' treating physicians via phone/fax. The subjects will receive a notification via the study web portal when their positive CMV test results are available with a link to a secure website that can be used to access them.

7.4 Questionnaires

7.4.1 Subject Questionnaire

Subjects who are enrolled and randomized into DBS arm will be asked to complete a questionnaire at three different time points throughout study participation: after the third training session, one month after discharge from transplant clinic, and at the end of study. The questionnaire will solicit user feedback on the utility and clarity of the DBS collection training, reminders, and shipping instructions. The questionnaire will be provided via web/mobile technology by Emmes.

7.4.2 Provider Questionnaire

Primary care providers of subjects who are enrolled and randomized into DBS arm will be asked to complete a questionnaire at the end of subject's study participation. The questionnaire will solicit the provider's feedback on the utility and quality of the DBS

testing. The questionnaire will be provided via fax by study coordinators at the time of last quarterly follow up records request.

7.5 Follow-up (Quarterly, ± 2 weeks)

Quarterly follow-up contacts will be done on all randomized subjects while enrolled in the study regardless of the randomization status. Study sites will follow up with subjects' primary care physicians to obtain the following records at each quarterly time point based on transplant date:

- Current immunosuppression
- Systemic steroid use
- Recommended CMV test interval (i.e., testing frequency)
- Office-based CMV test dates and results (i.e. PCR, IHC, histopathology, etc.)
- Interim hospitalizations
- CMV disease status
- Interim use of preemptive therapy for late CMV disease
- AEs
- Date of morphological relapse
- Survival status
- Problems with DBS or plasma kit integrity

If the scheduled quarterly visit is within 10 days of subject randomization, the corresponding quarterly visit will be waived.

7.6 Final Study Visit

At approximately 12 months after HCT (+4 months), study staff will conduct a closeout data collection of both randomized and observational cohorts by obtaining subject's medical records. The following information should be recorded on the appropriate electronic case report form:

- Current immunosuppression
- Systemic steroid use
- Recommended CMV test interval (i.e., testing frequency)
- Office-based CMV test dates and results (i.e. PCR, IHC, histopathology, etc.)
- Interim hospitalizations
- CMV disease status
- Interim use of preemptive therapy for late CMV disease

- AEs
- Date of morphological relapse
- Survival status
- Problems with DBS or plasma kit integrity

7.7 Hospitalizations

If a study subject is hospitalized, both the clinical trial coordinating center (Fred Hutch) and local site should be informed by the study coordinator or admitting physician, respectively. Subjects will be provided a letter that includes information about the study (i.e., study synopsis), which should be given to the admitting physician (refer to MOP). Information includes a short description of the study, instructions for hospital monitoring for CMV during the hospitalization, and contact information (phone, fax, and email).

If a DBS study participant is hospitalized, the participant should not collect weekly DBS, and local CMV monitoring with plasma CMV DNA PCR testing should be performed during the hospitalization.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Laboratory Evaluations

8.1.1 Clinical Laboratory Evaluations

8.1.1.1 Plasma CMV PCR Testing

Subject plasma samples will be tested for CMV viral load at the University of Washington Molecular Virology Laboratory using a validated qPCR assay that targets two CMV genes (i.e., UL55 and UL123) simultaneously using TaqMan probes, standard primers, and a single FAM fluorophore [40,41].

8.1.2 Special Assays or Procedures

8.1.2.1 CMV DBS PCR Testing

Subject DBS samples will be tested for CMV viral load at the University of Washington Molecular Virology Laboratory using a slightly revised and optimized version of the assay described in Limaye, et al [34]. In this assay, a single-use paper punch is used to punch a DBS from the Whatman 903 Protein Saver Card. DNA is extracted from the DBS (after washing) by adding 100µl of autoclave-sterilized 5% Chelax (Bio-Rad) and incubating at 95°C for 30 minutes (per manufacturer instructions). 20µl of eluted DNA are used for real-time TaqMan CMV PCR (as described in [40]) of a 50µl PCR mixture containing 25µl of 2× QuantiText multiplex PCR master mix (Qiagen) a 415 nM concentration of each primer (gB and IE-Ex4), and 100 nM concentration of the probes (gB and IE-Ex4).

8.1.3 Specimen Preparation, Handling, and Shipping

8.1.3.1 Specimen Collection and Transport to Processing Laboratory

DBS specimen:

Subjects are instructed to schedule same day or next morning (for evening DBS collection) mail carrier pick-up by website or phone or to deliver the envelope to the post office for overnight shipping with provided prepaid mailing envelopes. Subjects are to schedule pick up from Monday through Wednesday to avoid delays due to weekend shipping.

Plasma CMV PCR specimen:

Subjects are instructed to have 6ml EDTA drawn at their primary oncologist's office, primary transplant center, or blood draw facility, whichever is clinically scheduled, once their DBS is resulted positive or prior to initiation of preemptive therapy. The plasma sample will be sent from the collection center using the provided instructions and supplied prepaid mailing envelopes (which meet the biohazard shipping requirement) via overnight FedEx delivery. To ensure specimen's viability, processing laboratory must receive the sample within 48 hours of collection.

Detailed instruction regarding specimen collection and shipping will be provided to the subject at the time of training, prior to discharge from primary transplant clinic. All subjects will be provided with supplies with subject ID.

All specimens (both DBS and plasma) will be shipped to University of Washington Clinical Virology Laboratory (1616 Eastlake Ave E, Suite 320, Seattle, WA 98109) for analysis.

Specimen collection kits will be supplied by the coordinating center, Fred Hutch, to each site where they will be distributed to participating subjects.

8.1.3.2 Specimen Processing and Storage

Specimen processing and storage will occur at University of Washington Clinical Virology Laboratory. Samples will be labeled with date of collection, study protocol number, and subject ID. Leftover of plasma samples from EDTA tubes will be stored at -80 °C.

9 ASSESSMENT OF SAFETY

9.1 Adverse Events

AEs will be elicited from subjects in the DBS arm at quarterly follow-up contacts and at the final closeout contact.

For this trial, a reportable AE is defined as any clinically important untoward medical experience directly related to DBS collection, that is, the finger-stick procedure. The study source documents will include the assessment and evaluations of all AEs as defined above.

Only those events that are considered “Severe” and “Related” to the DBS collection and finger stick procedure will be reported. See below for definition of “Severe” and “Related”:

- Severe: Events interrupt a subject’s usual daily activity and require systemic drug therapy or other medically administered treatment.
- Related: There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

AEs as defined above are reported on appropriate CRFs in the AdvantageEDC^{SM}.

If an AE is considered “Unexpected”, the appropriate CRF must be submitted within 10 calendar days.

- Unexpected: Event is not listed in study materials or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the general investigational plan. For example, under this definition, an infection requiring antibiotic treatment and causing a missed day of work may be considered Expected. An infection causing an extended absence from work or leading to necrosis may be considered Unexpected.

DMID Medical Monitor will review adverse events and determine if additional reporting is applicable.

9.2 Serious Adverse Event of Special Interest (SAESI)

Breakthrough CMV disease events (mainly gastrointestinal disease) are expected to occur in both study arms at low frequency, however, in order to detect unusual patterns of

surveillance failure, breakthrough CMV disease will be considered a Serious Adverse Event of Special Interest. No other serious adverse events will be collected and reported in this trial.

Breakthrough CMV disease (only proven/probable, as defined in 3.2.2) events after start of surveillance testing will be considered a serious adverse event of special interest and will be reported within one working day of site awareness on an SAE form to the DMID Pharmacovigilance Group. Fax forms to 1-800-275-7619 (US) or email to PVG@dmidcroms.com. The DMID Pharmacovigilance Group may be reached at 1-800-537-9979 (US), if needed.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAESI by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAESI for potential impact on study subject safety and protocol conduct.

If the DMID Medical Monitor judges that there is a cluster of SAESI that represents a trend or suspicious pattern of SAESI, the DSMB will review those events in an ad-hoc meeting.

In addition, following an evaluation of SAESIs of breakthrough CMV disease, DMID shall report the results of such evaluation to FDA and participating clinical site investigators within 10 working days after DMID first receives notice of SAESI evaluation from the DSMB. Clinical site investigators and Coordinating Center shall report to respective IRBs according to respective IRB guidelines.

9.3 Unanticipated Problems

Investigators must report Unanticipated Problems, regardless of severity, associated with self-administered finger-stick DBS collection or assay. This includes false positive or false negative assay results. An Unanticipated Problem is defined as:

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied

- Related or possibly related to participation in the research, in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research

The investigator shall submit to DMID and to its local IRB a report of any Unanticipated Problem occurring during an investigation as soon as possible, but in no later than 10 working days or per local guidelines, respectively, after the investigator first learns of the effect.

DMID as the sponsor shall immediately conduct an evaluation of any Unanticipated Problem. If an Unanticipated Problem presents an unreasonable risk to subjects, DMID shall terminate all investigations or parts of investigations presenting that risk as soon as possible. Termination shall occur no later than 5 working days after the sponsor makes this determination and no later than 15 working days after the sponsor first received notice of the effect. Resumption of any termination will not occur without FDA and IRB approval. (21 CFR 812.46)

If DMID conducts an evaluation of an Unanticipated Problem under 21 CFR 812.46 (b) then DMID will report the results of such evaluation to FDA and to all participating investigators no later than 15 working days after DMID first receives notice of the effect. Clinical site investigators and Coordinating Center shall report to IRBs per local guidelines after receipt of evaluation from DMID. Thereafter DMID will submit such additional reports concerning the effect as FDA requests.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Information on finger-prick related adverse events will be obtained from the quarterly chart abstractions.

9.5 Safety Oversight

9.5.1 Data and Safety Monitoring Board (DSMB)

The DMID DSMB is convened by authority of DMID and is advisory to DMID and the study team. The DMID DSMB must consist of at least three voting members including a biostatistician experienced in statistical methods for clinical trials and a clinician with relevant expertise. Selection of DMID DSMB members should include consideration of clinical trials experience, relevant expertise, prior DSMB service, and absence of significant conflict of interest. DMID is responsible for deciding whether consultancies or the financial interests of the members materially affect their

objectivity. Members will notify DMID promptly if a change occurs that may create a potential conflict of interest.

The DMID DSMB will operate under the rules of a DMID-approved charter that will be reviewed and finalized following the organizational meeting of the DSMB. The DMID DSMB Charter serves as the standard operating procedure and defines the primary responsibilities of the DSMB, its membership, the purpose and timing of its meetings, data to be reviewed, and procedures for ensuring confidentiality and proper communication. The following times are the proposed scheduled meetings of the DMID DSMB:

- Organizational DMID DSMB meeting –The first meeting of the DMID DSMB will be primarily organizational and to review the study protocol, safety data shells, and frequency of scheduled meetings. This meeting will occur prior to the initiation of study enrollment.
- Interim analysis data review meeting- Among the first 50 subjects enrolled on the DBS intervention arm and followed for at least a year, if the lower one-sided 90% confidence interval for the percent of subjects experiencing CMV disease by one-year exceeds 8% (operationally 7 out of 50), a DSMB meeting will be convened. The DSMB will be charged with determining whether this exceptionally high rate of CMV disease has plausible clinical explanations related to the subject characteristics, or if it is the result of a failure in the monitoring system, and if so, what the source of the failure is (lack of compliance, failure of the system). Ultimately, they will make a recommendation to the PI as to whether any aspect of the study should be modified or terminated.
- Ad Hoc meeting to review safety events such Unanticipated Problems or SAESIs (breakthrough CMV disease reports).
- Final DMID DSMB meeting - 6 to 8 months after clinical database lock to review the cumulative safety data for the study. The data will be provided in a standard summary format. The DSMB may be asked to provide recommendations in response to questions posed by DMID.

The DMID DSMB may also be convened for an *ad hoc* meeting, an unplanned meeting that is called for a specific purpose such as when a study halting rule is met. The meeting can be requested by any party with the responsibility of overseeing the trial (such as the PI, DSMB, DMID). In the case of an *ad hoc* meeting, the DMID DSMB may request special reports on an as-needed basis.

If the study is discontinued, follow-up visits for safety would continue.

10 MONITORING

10.1 Study Kit Monitoring

The clinical sites are responsible for verifying the integrity of the study kit before supplying subjects with kits at enrollment and resupplies. Both the DBS and Confirmatory Whole Blood kits will be verified. The subject will be instructed to report problems with study kit integrity to the clinical site. If a problem is identified with study kit, the subject will contact the site, which will record problems on the appropriate eCRF. The subjects will submit resupply requests of additional study kit through the web/mobile technology.

The SDCC will perform data logic checks and queries on the data entered into the data collection system.

11 STATISTICAL CONSIDERATIONS

11.1 Study Outcome Measures

11.1.1 Primary Endpoint

The primary endpoint will be the proportion of subjects who complete >90% of their recommended CMV monitoring tests by 1-year post-transplant. We will use logistic regression modeling to compare this proportion between subjects randomized to DBS testing versus standard office-based testing. Since randomization will be stratified by transplant site and subject's perceived ease of access to blood draw facility, these factors will be used as adjustment factors in the models.

For each subject, the number of CMV monitoring tests performed will be assessed and the percent of recommended tests will be calculated. Subjects with percent completion >90% will be considered a success with regard to the primary endpoint. The person-specific denominator for the proportion, number of expected CMV monitoring tests, will be variable and dependent on subjects' immunosuppression and hospitalizations during the study. For example, subjects in this study that are highly immunosuppressed a weekly monitoring schedule is generally recommended, often throughout the first year after transplantation. If such a subject were discharged from the primary transplant center at day 100, there would be 38 remaining tests until day 365 after HCT (if the subject is still alive at 1 year). We will assess the actual number of tests performed and calculate the percent of tests (e.g. 36/38 = 94.7%). Another scenario would be for a subject that stopped monitoring and was then re-started on high-dose steroids for treatment of GVHD with resumption of weekly testing, resulting in yet another denominator. Study participants in the DBS arm who are hospitalized during the study should not collect DBS samples while admitted. Rather, admitting hospitals should monitor participants for CMV with plasma CMV DNA PCR testing as recommended for their level of immunosuppression. Those inpatient testing timepoints will not be included in the person-specific denominator.

The primary analysis will be an intent-to-treat (ITT) analysis of all subjects randomized. Another key analysis will be the modified intent to treat analysis of all subjects who had at least one test done after discharge from the primary transplant team.

11.1.2 Secondary Endpoint

As a secondary endpoint we will be comparing the incidence of late CMV disease by one year post HCT between the two randomized groups using competing risk regression with death treated as a competing risk event [42]. Using similar methods, we will also compare the CMV disease incidence of trial participants compared to subjects who received HCTs at the clinical sites in the similar time period but did not participate in the trial. As expected, the planned trial would not be sufficiently powered to detect a difference in late CMV disease incidence from 8% (current standard care) to 2% (observed in clinical trial). A trial powered for that detectable effect size would require 330 subjects to have 80% power (one-sided p values 0.05).

To determine the rate of CMV disease at participating sites during the clinical trial we will collect concurrent retrospective baseline data as well as CMV disease and survival data from subjects that were eligible for randomization but decided not to participate (the majority of subjects return to the transplant center at one year). The purpose of this analysis is to determine whether the subjects who participated in the trial are representative of the population at risk for late CMV disease and whether the phenomenon of late CMV disease continues to occur throughout the study period.

To assess the correlation of CMV viral load between DBS and plasma samples, we will use linear model. Other outcomes will be tabulated and compared between groups using Chi-Square, Fisher's exact, and log rank tests, as appropriate.

The relative contribution of mobile phone reminders, subject adherence to the device, and failures of the testing kit itself will be analyzed among subjects randomized to the DBS arm who did not reach the 90% adherence threshold.

11.2 Sample Size Considerations

Power calculations utilized a binomial distribution to ascertain the number of subjects needed to detect a clinically meaningful difference in proportions of subjects meeting the primary endpoint [43]. Based on our preliminary data, we assumed the proportion of individuals completing >90% of their recommended surveillance tests in the standard office-based testing arm would be in the range of 50-55%. In our previously reported clinical trial, high rates of CMV monitoring completion resulted in the clinically relevant outcome of a low incidence of late CMV disease [22] . To be successful, this proof of concept trial needs to demonstrate that similarly high rates of monitoring can be achieved with subject collected DBS monitoring. With 150 subjects, randomized 2:1, we will have 90-96% power to detect an absolute difference in testing rates of 25-30% between subjects in the intervention (80% adherence) and control arms (50% adherence)

(Table 2). Although the 2:1 randomization does not result in increased statistical power, randomizations that favor the experimental arm (2:1 or 3:1) appear to increase the appeal to the potential study participant and have been used successfully in several recent multicenter CMV randomized trials [24,25,44].

Table 1 Power calculations for primary analysis

Sample size Total	Sample size DBS group	Sample size Control	Proportion of subjects with >90% Adherence rate DBS arm	Proportion of subjects with >90% Adherence rate Control arm	Sig. level Two-sided	Power
150	100	50	0.8	0.5	0.05	**96%**
150	100	50	0.8	0.55	0.05	**89%**
150	100	50	0.8	0.58	0.05	**80%**

11.3 Participant Enrollment and Follow-Up

Study is expected to enroll 150 allogeneic HCT recipients who meet inclusion criteria for randomized cohort and 450 allogeneic HCT recipients who meet inclusion criteria for observational cohort over period during which randomization is performed (and one year prior if needed) between four study sites. Follow-up for is described in the time and events schedule in Appendix C.

11.4 Randomization Scheme

Randomization will be done by the SDCC online using the enrollment module of AdvantageEDC^{SM}. Subjects will be stratified at the time of randomization according to transplant site and subject's perceived ease of access to blood draw facility in order to prevent major imbalances within the study.

11.5 Analysis Plan

As described under Primary Endpoint, the primary analysis will be a logistic regression analysis, comparing the study arms in an Intent-to-Treat analysis including all randomized subjects. Prior analyses will include careful checks of all key data elements (primary and secondary endpoints and subject characteristics) to ascertain implausible and missing values. Reports including all missing and implausible values will be generated and elements will be checked with source documents to attempt to correct or replace those values. If a data value is considered to be completely implausible and cannot be corrected with available information, its value will be considered "missing". Once these edits have iterated until no further corrections can be made, each data element will be summarized by study arm, and percent of missing data evaluated. Ideally, missing data will be negligible in this study, but if that is not the case, we will determine the ideal method for handling this to avoid biased results (e.g. inverse probability weighting,

multiple imputation, etc.). Our final analysis plan will be reviewed prior to data lock and amended as needed before the start of analysis.

The primary study period for subjects that have been withdrawn from the study will be from randomization to the day of withdrawal. A sensitivity analysis will be performed to estimate the impact of withdrawal on the primary endpoint using the maximum possible study period.
The study statistician may provide an analysis of aggregate, uncleaned data from the observational cohort on the estimates of late CMV disease and on the sensitivity of CMV detection by PCR in DBS vs plasma samples to the investigators and sponsor for the purpose of abstract preparation or presentation or planning for future studies. While the results provided will not be used to make any decisions about the conduct of this study, they may be used to make decisions on activities external to this trial such as the design of other studies on CMV disease in transplant recipients or on CMV detection methodologies.

11.5.1 Interim Safety Monitoring

Among the first 50 subjects enrolled on the DBS intervention arm and followed for at least a year, if the lower one-sided 90% confidence interval for the percent of subjects experiencing CMV disease by one-year exceeds 8% (operationally 7 out of 50), a DSMB meeting will be convened. The DSMB will be charged with determining whether this exceptionally high rate of CMV disease has plausible clinical explanations related to the subject characteristics, or if it is the result of a failure in the monitoring system, and if so, what the source of the failure is (lack of compliance, failure of the system). Ultimately, they will make a recommendation to the PI as to whether any aspect of the study should be modified or terminated.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms, most of which serve as source documents for this study, will be derived from the electronic CRF and provided by the SDCC to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF derived from the data collection forms should be consistent with the source documents or the discrepancies should be explained.

The sponsor will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, monitoring, and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, records of receipt, use or disposition of the device, records of each subject's case history and exposure to the device, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the laboratories, and medico-technical departments involved in the clinical trial. Data collection forms used as source documents will be derived from the eCRFs and be provided by the SDCC.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the investigational site is responsible for conducting routine quality assurance and quality control activities to internally monitor study progress and protocol compliance. The PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The PI will ensure all study personnel are appropriately trained and applicable documentation is maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site principal investigator (PI) will ensure that this study is conducted in full conformity with principles of The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [April 18, 1979]) and codified in 45 CFR 46, 21 CFR 50 and 21 CFR 56, as applicable. The PI will also ensure conformity with the ICH E6; 62 Good Clinical Practice and applicable federal regulations, guidance, and Guidelines for Good Clinical Practice and Clinical Trials with humans.

14.2 Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and informed consent form will be reviewed and approved by the appropriate IRB listed on its FWA.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to DMID. The IRB FWA number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site principal investigator for submission to the IRB.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate. If an in-person informed consent cannot be obtained, remote informed consenting via video teleconferencing is permissible under the site's IRB approved remote consenting policy (See MOP for details and instructions).

An investigator or designee will describe the protocol to potential subjects in person. The key information about the purpose of the study, the procedures and experimental aspects of the study,

the risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research and a detailed summary of the proposed study procedures and study interventions/products. This will include the aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, and all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject that are currently unforeseeable). The expected duration of the subject's participation in the trial, alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures will also be included in the detailed explanation.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the subject is authorizing such access.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed Consent forms will be IRB-approved and subjects will be asked to read and review the consent form. Subjects must sign the informed consent form prior to starting any study procedures being done specifically for this trial.

Once signed, a copy of the informed consent form will be given to the subjects for their records. The subjects may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study personnel may employ recruitment efforts prior to obtaining study consent if a subject-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a subject-specific screening consent on record, site clinical staff may pre-screen via chart review and refer potential subjects to the Research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

All subjects must sign an informed consent form that complies with the requirements of both 21 CFR 50 and 45 CFR 46.

Subjects will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them. The consent form must not include any exculpatory statements.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

Investigators will follow the site IRB/IEC requirements for enrollment of minors in this study. In most cases, investigators or designee will conduct the consent process with the minor and their parent(s)/legal guardian, who will be given an IRB/IEC-approved permission form, which may be referred to as a consent form, to read, review, and sign prior to any study procedures. The consent process will be conducted as outlined above in section 14.3, with both the minor subject and the parent(s)/legal guardian signing the consent form.

The investigator or designee will describe in simplified terms the details of the study intervention/product, study procedures, risks and discomforts, benefits, and other consent elements, as appropriate.

No specific assent will be used in this study; subjects ≥15 and <18 years of age will sign consent together with his or her parent or guardian. When a minor subject who reaches the age of majority (18 years of age) during the study period, the subject will be re-consented without a parent or guardian's signature at the next visit prior to study procedures. When no further visits are planned but the subject's participation is ongoing, requirements for reconsent will follow local IRB/IEC-approved processes.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This study will be inclusive of all adults who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background. Children between ≥15 and <18 years of age are also included. Children < 15 years of age are excluded because of requirements for self-reporting and self-administration of study procedures.

14.5 Subject Confidentiality

This research is covered by a Certificate of Confidentiality from the NIH. Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. The investigators and their staff may not disclose or use information documents or biospecimens that may identify the subjects in any federal, state, or local civil, criminal, administrative, legislative or other action, or be used as evidence unless the subject has consented. This does not apply to requests for information from the NIH or its representatives that are needed to monitor or audit the study, or for information that must be disclosed in order to meet FDA requirements.

At the clinical trial coordinating center, multiple mechanisms have been established to protect the confidentiality of specimens, medical records, and data. All personnel who work on this study must sign a pledge of confidentiality across all participating sites. Access to the database is controlled through secure password protection, and passwords must be changed quarterly. Access to the work site is controlled through passkeys and ID badges. Individuals who are not employees must be escorted at all times by an employee.

Study sites employ site-specific confidentiality measures, including electronic and physical barriers.

Each participating subject will be assigned an identification number to be used for all subject data. Links to subject name and identifiers will be maintained and stored in files on computers protected by password and in locked office cabinets. All clinical data collected at individual sites will be entered into CRFs on a secure web-based system.

Specimens will be coded with unique study identification numbers in order to ensure subject confidentiality. No identifying information of any kind may be released to persons or agencies without specific written permission.

No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences.

The Sponsor, it's designee, or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the subjects, and records of receipt, use or disposition of the device in this study. The clinical study site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

14.6 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining specimens for possible use in future research studies. Residual specimens will be stored indefinitely at Fred Hutch. Before conducting additional research unrelated to the original research questions, the Fred Hutch IRB will review the request and decide whether the request meets minimal risk requirements or whether additional specific consent is required. The Fred Hutch IRB serves as central IRB for this issue. Subjects may opt out of future research unrelated to the original research question.

Samples may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. Tests may include genetic research to look at things such as which genes affect infection or how genes work. Each sample will be encoded (labeled) *only* with a barcode and a unique tracking number to protect subject's confidentiality.

There are no benefits to subjects in the collection, storage, and subsequent research use of specimens. Reports about future research done with a subject's samples will NOT be kept in

their health records. Subjects can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A subject's decision can be changed at any time up to the point the specimens are released for research use by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood has already been used for research purposes, the information from that research may still be used.

14.7 Disclosure of Individual Research Information

In this protocol, we intend to provide each DBS arm participant with his/her specific DBS and plasma CMV test results sent to and obtained from central laboratory. In addition, we intend to provide each participant with an overall summary of the study results at the end of the study.

15 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

This study will be inclusive of all adults who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background. Children between 15 and 18 years of age are also included.

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Company, LLC will serve as the Statistical and Data Coordinating Center for this study and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including AEs, concomitant medications, and CMV disease status) and clinical laboratory/virologic data will be entered into a 21 CFR Part 11-compliant Internet Data Entry System (IDES) provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

15.3 Types of Data

Data for this study will include virologic and clinical data.

15.4 Timing/Reports

Reports on subject's compliance with the testing algorithm will be generated semiannually in order to assess overall study conduct. DSMB reports will be generated as previously described in section 9.5.1.

15.5 Study Records Retention

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects.

Records and documents pertaining to the conduct of this study, including CRFs, data collection forms, other source documents, consent forms, and laboratory test results, must be retained by the investigator for at least 2 years after the date that the records are no longer required for supporting a premarket approval application; or until 2 years after the investigation discontinued and FDA has been notified. No study records will be destroyed without prior authorization from DMID. Informed consent forms for future use will be maintained as long as the samples exist.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1, and 5.20.2.

For this protocol, since adherence to surveillance testing is a key outcome of the study that will be captured as an endpoint, the lack completion of scheduled surveillance tests (including confirmatory tests at the physician's office or blood draw facility) or inadequate sample quality will **not** be recorded as protocol deviations but will be captured on case report forms.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported via The Emmes Corporation's IDES. All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form (IDES form) must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

16 IDE REPORTING REQUIREMENTS AND PROCEDURES

16.1 Reporting to FDA, investigators and IRBs for Study Conducted Under IDE

DMID will comply with all FDA reporting requirements and timelines as outlined in 21 CFR 812.3(s), 812.46 and 812.150. In addition to safety reporting as outlined in Section 9.0 of this protocol, DMID will report the following:

- Within 5 working days of receipt of notice or becoming aware of the event, DMID will notify FDA and all participating investigators responsible for reporting to local IRBs of the following: withdrawal of approval of the study by a reviewing IRB or use of the device by an investigator without first obtaining consent.

- Within 5 working days of receipt of any withdrawal of approval by FDA, DMID will notify all participating investigators responsible for reporting to local IRBs.

- Within 30 days of receipt of a request by any investigator to return, repair, or otherwise dispose of any units of the device, DMID will notify FDA and all participating investigators responsible for reporting to local IRBs of the request and reasons for that request.

- DMID will report annually to the FDA and all participating investigators on the progress of the study and will report a list of current investigators on the study to FDA at 6 month intervals.

16.2 Investigator required reporting

In addition to safety reporting as outlined in section 9.0 of the protocol, the investigators will report to DMID, within 5 working days the following:

- Any use of the device without first obtaining informed consent
- Withdrawal of approval by the investigator's reviewing IRB.

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17 PUBLICATION POLICY

Following completion of the study, the results may be published in a scientific journal or presented at a scientific symposium or congress. All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication.

All clinical trials supported by the NIH must be registered on ClinicalTrials.gov no later than 21 days after enrollment of the first subject. This study will be registered in accordance with this policy by DMID, as the Sponsor and responsible party for this trial. Results will be submitted within 12 months following the primary completion date of the study, unless a waiver for delayed posting is granted. As part of this posting, a copy of the protocol and the Statistical Analysis Plan will also be posted.

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APPENDIX A: DBS CMV PCR ASSAY COMPARISON

Description	Procedure in Publication	Current Procedure
Filter paper used	Whatman FTA Elute cards	Whatman 903 Protein Saver cards
Size of each punch	3 mm in diameter	6 mm in diameter
Number of punchs used for DNA extraction	4	2
PCR master mix	QuantiTect Muliplex PCR mix with ROX	QuantiTect Muliplex PCR NoROX mix
PCR Machine	7900HT sequence detection system	QuantStudio 7 flex real-time PCR system
IU conversion	1 IU= 4 copies (AcroMetrix)*	1 IU=1.4 copies (WHO)
Internal control template	Spiked into PCR master mix	Spike into extraction lysis buffer
Internal control primers and probe	EXO	EXO BS
DNA Elution volume	100 ul	100 ul
Amount of DNA used for each PCR	20 ul	20 ul
CMV primers and probe	gB and IE-EX4	gB and IE-EX4
CMV PCR standard	1e1 to 1e5	1e1 to 1e5

APPENDIX B: DEFINITION OF END-ORGAN CMV DISEASE

CMV Disease	Clinical Signs and Symptoms	Proven	Probable	Note
Pneumonia	Pneumonia (e.g. new infiltrates on imaging, hypoxia, tachypnea and/or dyspnea)	Clinical signs and symptoms with CMV in lung tissue by virus isolation, rapid culture, histopathology, IHC, or DNA hybridization	Clinical signs and symptoms with detection of CMV by viral isolation, rapid culture of BALF, quantitation of CMV DNA in bronchoalveolar lavage fluid (BALF)	Quantitative PCR on lung tissue could be defined as "possible"
Gastrointestinal (GI) disease	Upper and/or lower GI symptoms	Clinical signs and symptoms with macroscopic mucosal lesions plus CMV documented in tissue by virus isolation, rapid culture, histopathology, IHC, or DNA hybridization	Clinical signs and symptoms with CMV documented in tissue but without the requirement for macroscopic mucosal lesions	CMV documented in blood by NAT (PCR) or Ag or PCR from tissue is not sufficient for CMV GI disease diagnosis. These could be defined as "possible".
Retinitis	Retinitis	Judged by an ophthalmologist	Not used	Diagnosis could be supported by vitreous fluid by NAT (PCR)
Encephalitis/ Ventriculitis	Central nervous system (CNS) symptoms	Clinical signs and symptoms with CMV documented in tissue by virus isolation, rapid culture, histopathology, IHC, or DNA hybridization	Clinical signs and symptoms with CMV documented in in CSF without visible contamination plus abnormal imaging or EEG	Not used
Other Organs [35]	Most organs	Clinical signs and symptoms with CMV documented in tissue by virus isolation, rapid culture, histopathology, IHC, or DNA hybridization	Not used	Not used

APPENDIX C: SCHEDULE OF EVENTS

Visit/Contact	Screening[1]	Enrollment[2]	Initial Study Visit[3]				DBS self-collection	Plasma Collection[4]	Quarterly Contact 1	Quarterly Contact 2	Quarterly Contact 3	Final Study Visit
			Training 1[5]	Training 2[6]	Training 3[7]	Discharge						
Days after HCT	60-180						60-365	60-365	90	180	270	365
Window (days)	N/A								±14	±14	±14	(+120)
Confirm informed consent		X										
Eligibility assessment	X, Z											
Randomization		X										
Review of medical history		X, Z										
Review of concomitant medication		X, Z				X			X	X	X	X, Z
Confirm CMV monitoring interval						X, Z			X	X	X	
Adherence counseling[8]		X										
DBS collection and website training[9]			Y	Y	Y		Y[10]					
Provision of initial study kits						Y						

[1] It is recommended to have screening done approximately one month prior to planned discharge from the primary transplant clinic
[2] Screening, enrollment, and initial study visit (trainings for DBS arm) may occur on the same day as long as all trainings are completed by subjects with proficiency and confirmed by study personnel prior to discharge from primary transplant clinic.
[3] Trainings 1-3 must be completed prior to planned discharge from the subject's primary transplant clinic.
[4] Confirmatory plasma sample will be collected from DBS arm subjects if they had a CMV positive DBS.
[5] Training with study staff supervision.
[6] Training with study staff supervision.
[7] Training without study staff supervision.
[8] Adherence counseling should be done on all randomized subjects after enrollment but prior to discharge from primary transplant clinic.
[9] DBS collection is required at each DBS training visit. Website and mobile technology training may be optional for training 2 and 3 as necessary.
[10] DBS collection starts the week after discharge and ends approximately one-year post-HSCT

Visit/Contact	Screening[1]	Enrollment[2]	Initial Study Visit[3]				DBS self-collection	Plasma Collection[4]	Quarterly Contact 1	Quarterly Contact 2	Quarterly Contact 3	Final Study Visit
			Training 1[5]	Training 2[6]	Training 3[7]	Discharge						
Days after HCT	60-180						60-365	60-365	90	180	270	365
Window (days)	N/A								±14	±14	±14	(+120)
Kit integrity monitoring[11]						Y	Y					
Subject questionnaire[12]						Y	Y					Y
Provider questionnaire[13]												Y
Safety monitoring									X	X	X	X
Obtain interim medical records[14]									X	X	X	X, Z

X = performed for all randomized subjects

Y = performed for DBS arm subjects only

Z = performed for observational cohort only

GRAY columns may not be required. Depending on when a subject enrolls in the study, the duration of participation will be between 26 and 44 weeks. Columns marked in GRAY apply to those subjects whose participation includes these time points. If the scheduled quarterly visit is within 10 days of subject randomization, the corresponding quarterly visit will be waived.

[11] Subjects are instructed to report of any failures in DBS kits during the duration of study participation. At each time of subject reporting, study staff is responsible for uploading the reported information to Advantage eClinical eCRF. If reported problem meets adverse (AE) criteria, a separate AE form must be submitted.

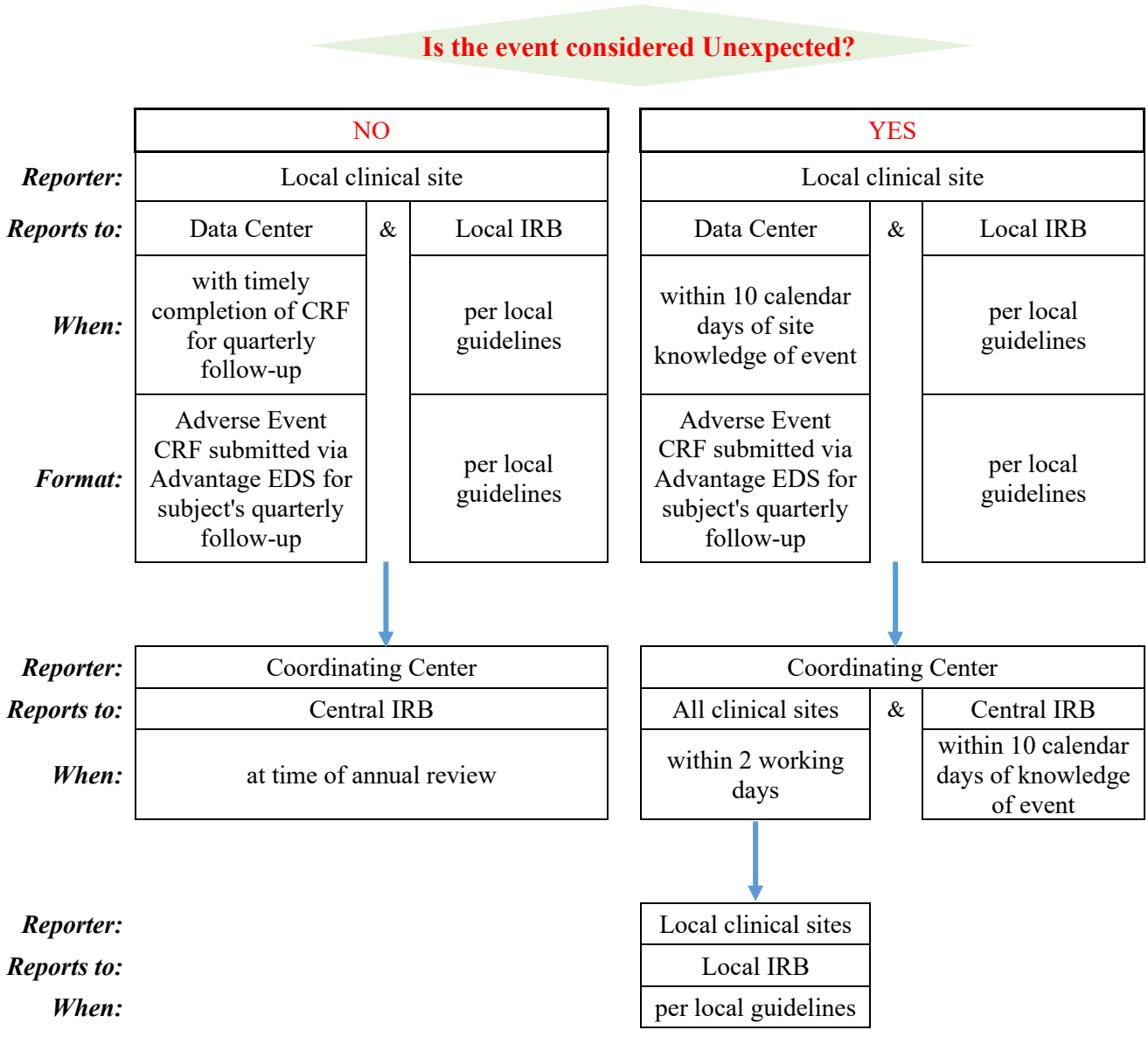
[12] Patient questionnaire will be given after 3rd training session, 1 month after discharge, and at the end of study.

[13] Primary care providers will be given a questionnaire at the time of last quarterly contact for medical records review.

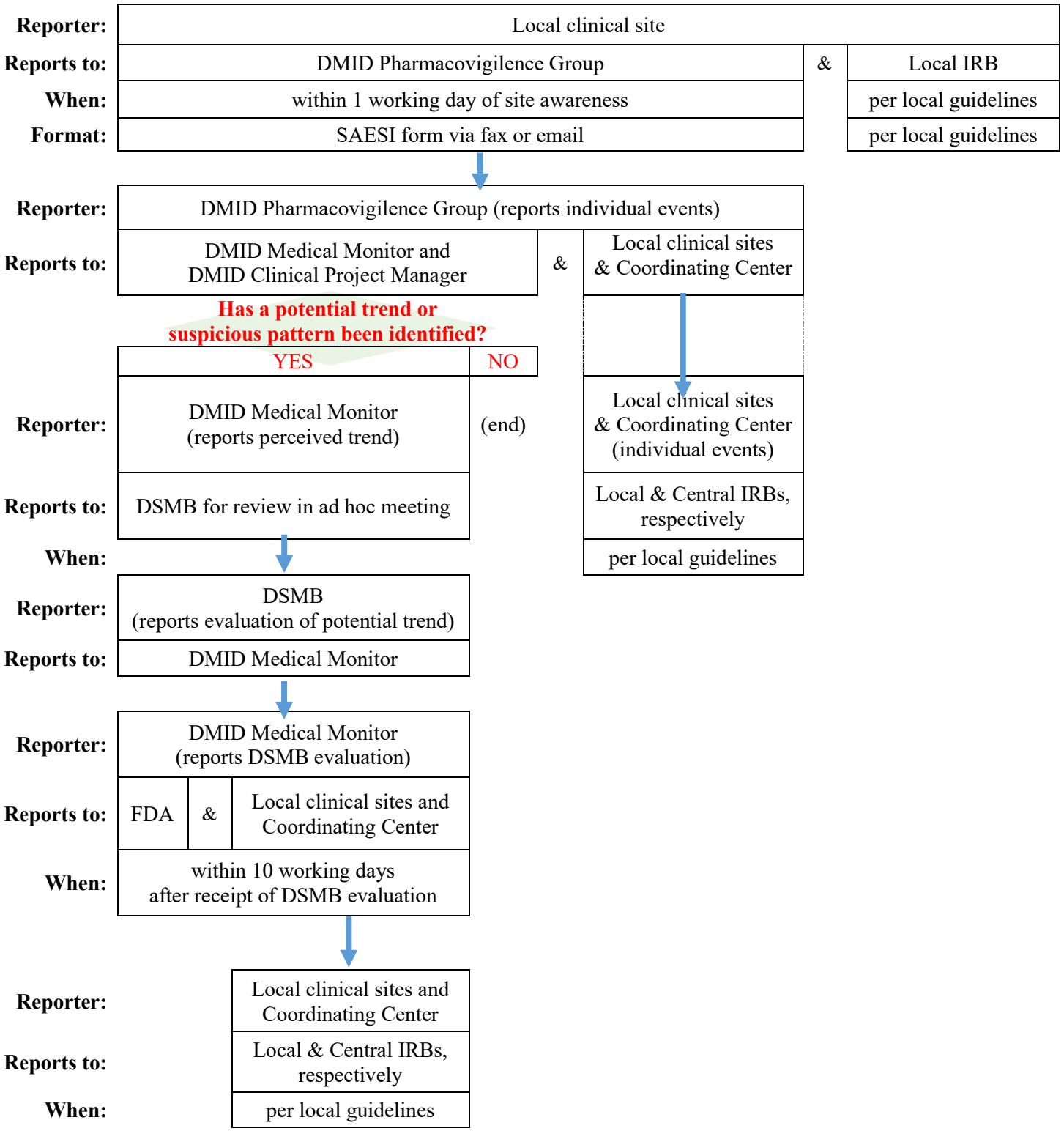
[14] Obtain CMV testing frequency and results, presence of CMV disease, use of antivirals of CMV treatment, and use of steroids for GVHD

APPENDIX D: SAFETY REPORTING

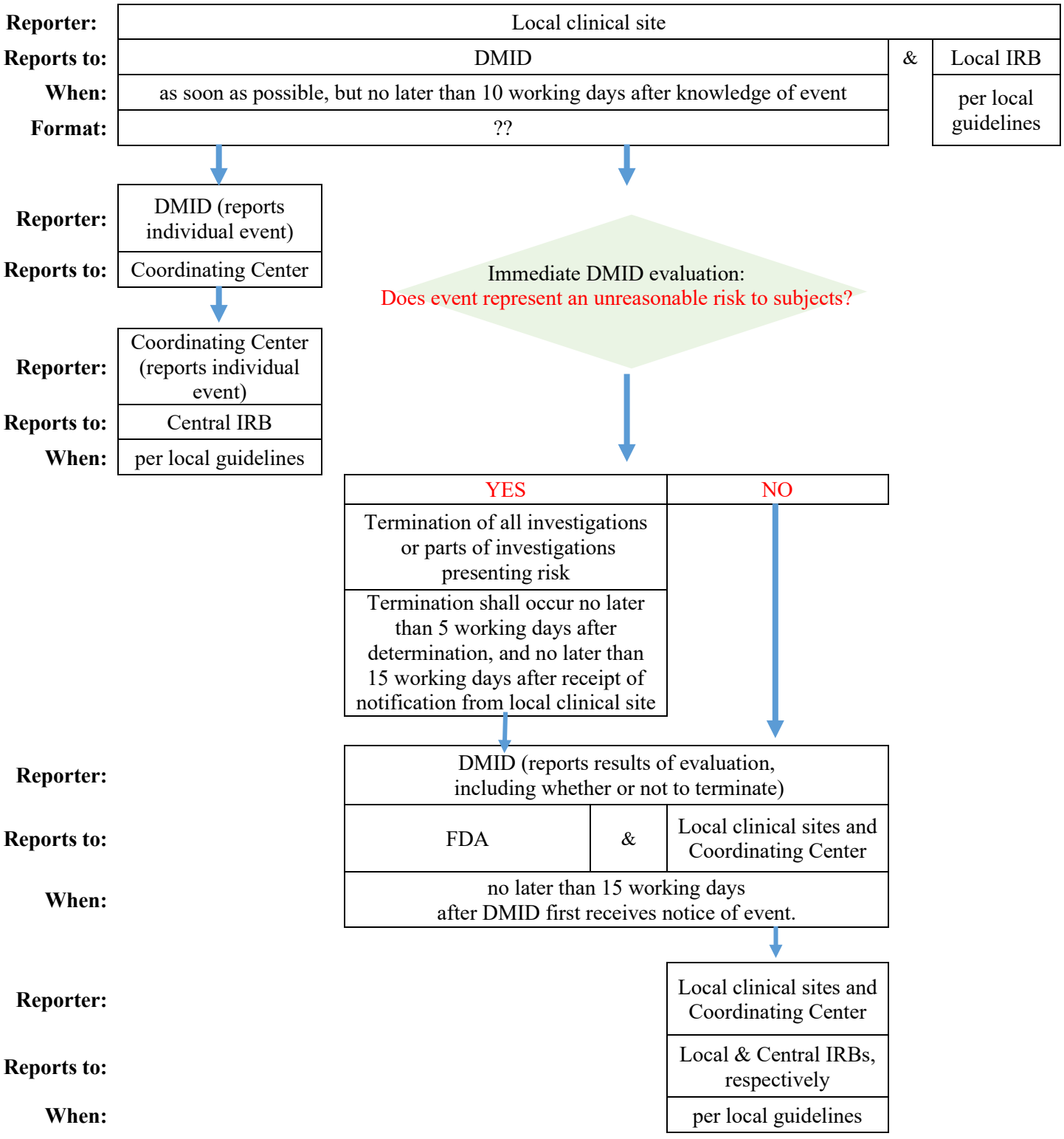
1. Adverse Event



2. Serious Adverse Event of Special Interest



3. Unanticipated Problems


Reporter:
Local clinical site
Reports to:
DMID
&
Local IRB
When:
as soon as possible, but no later than 10 working days after knowledge of event
per local guidelines
Format:
??
Reporter:
DMID (reports individual event)
Reports to:
Coordinating Center
Reporter:
Coordinating Center (reports individual event)
Reports to:
Central IRB
When:
per local guidelines
Immediate DMID evaluation:
Does event represent an unreasonable risk to subjects?
YES
NO
Termination of all investigations or parts of investigations presenting risk
Termination shall occur no later than 5 working days after determination, and no later than 15 working days after receipt of notification from local clinical site
Reporter:
DMID (reports results of evaluation, including whether or not to terminate)
Reports to:
FDA
&
Local clinical sites and Coordinating Center
When:
no later than 15 working days after DMID first receives notice of event.
Reporter:
Local clinical sites and Coordinating Center
Reports to:
Local & Central IRBs, respectively
When:
per local guidelines