

## SUPPLEMENTARY APPENDIX

### **Multicenter Study of Banked Third Party Virus-Specific T-Cells to Treat Severe Viral Infections after Hematopoietic Stem Cell Transplantation**

Ann M Leen<sup>1</sup>, Catherine M Bollard<sup>1</sup>, Adam M Mendizabal<sup>2</sup>, Elizabeth J Shpall<sup>3</sup>, Paul Szabolcs<sup>4</sup>, Joseph H. Antin<sup>5</sup>, Neena Kapoor<sup>6</sup>, Sung-Yun Pai<sup>5,7</sup>, Scott D. Rowley<sup>8</sup>, Partow Kebriaei<sup>2</sup>, Bimalangshu R Dey<sup>9</sup>, Bambi J Grilley<sup>1</sup>, Adrian P Gee<sup>1,10</sup>, Malcolm K Brenner<sup>1</sup>, Cliona M Rooney<sup>1,10</sup> and Helen E Heslop<sup>1</sup>.

#### **Table of Contents**

Supplementary Table 1: Characteristics of Virus-specific T-cell Lines
Supplementary Table 2: Outcome of Patients with No Matching Line
Supplementary Figure 1. Study Flow Chart
Supplementary Figure 2. CMV: Immune reconstitution
Supplementary Figure 3. AdV: Immune reconstitution
Supplementary Figure 4. EBV: Immune reconstitution
Supplementary Figure 5. Detection of VST -line derived V $\beta$ TCR sequences in recipient blood
Legends for Supplementary Figures
Clinical Protocol

**Supplemental Table 1: Characteristics of Virus-specific T-cell Lines**

Line No.	Patients to Whom Given	HLA Phenotype			Specificity (SFC/1x10 <sup>5</sup> )			Phenotype (%)						
		A	B	DR	Adv	CMV	EBV	CD3	CD4	CD8	CD3/RO+/CD62L-	CD3/RO/CD62L+	CD3/56+	CD3-/56
C2274	16; 20	11, 24	35, 44	13, 15	529	451.5	164.5	98.83	1.49	95.88	94.94	1.94	11.41	0.77
C3366	19; 27; 49	24, 32	13, 35	04, 07	126	593	163	98.11	12.41	87.02	99.09	1.94	4.39	1.77
C2270	19; 70	01, 24	51, 57	03, 05	529	856	206	98.56	5.36	92.83	95.97	1.34	3.65	1.26
C3629	24; 56; 69; 85;97	02, 24	08, 14	01, 03	241	857	144	98.33	8.23	88.93	87.13	9.49	8.08	1.36
C2420	22; 23; 61	01, 80	07, 53	11, 15	144	48	377	99.54	98.84	0.03	73.08	24.85	5.86	0.4
C3365	30; 62; 74	03, 11	15, 51	10, 15	24.5	238	169	99.82	17.56	79.43	66.66	30.3	44.5	0.11
C2582	18	03, 68	40, 58	04, 04	82	648	186	97.63	24.18	74.24	58.25	37.54	7.82	0.95
C2339	21; 40; 47; 58; 86; 90	02, 68	35, 40	04, 04	182	176	43.5	99.27	5.25	92.5	92.1	4.27	6.74	0.55
C3466	25; 67	01, 03	47, 49	03, 13	385	984	348	97.43	8.63	85.32	5.59	89.15	15.5	2.3
C3528	26; 57	02, 24	35, 52	08, 08	624.5	101	253.5	98.85	39.25	55.34	68.47	23.24	17.42	0.94
C3617	37; 79; 94	31, 33	15, 58	03, 15	8.5	Seroneg	5054	99.75	99.09	0.72	58.8	39.68	1.55	0.07
C3005	38; 43; 81	02, 02	08, 49	03, 13	238	990	131	99.2	5.3	83.64	40.23	52.34	9.58	0.86
C2353	41; 93; 96	01, 02	08, 15	03, 12	93	2189	183	97.72	4.7	92.67	94.4	3.49	6.52	1.96
C3889	50; 52; 68; 71; 83; 84	01, 24	08, 58	03(17)	836	386	300	95.76	9.67	79.79	4.67	86.16	4.11	9.65
C3874	73	02, 03	35, 46	04, 09	360	300	30	96.58	12.27	85.05	33.68	64.66	2.42	2.77
C2457	59; 76	03, 68	35, 57	07, 14	585	699	400	96.09	10.54	82.62	64.46	27.52	13.62	3.68
C3921	77; 87; 91	02, 02	27, 51	01, 03	44	3716	116	99.4	5.41	93.2	68.95	30.05	16.08	0.46
C4378	88	03, 03	07, 07	15, 15	133	Seroneg	67	95.68	89.71	4.35	3.21	90.08	0.29	3.95

**Supplementary Table 2 Outcome in Patients without Matching Line**

<b>Patient ID</b>	<b>Infection Cohort</b>	<b>Viral Outcome</b>
000017	Adenovirus	Continued Cidofovir and Foscarnet - died of progressive adenovirus infection
000031	Adenovirus and CMV	Died of progressive adenovirus pneumonia
000039	CMV	Received Foscarnet but still had elevated CMV levels in blood by PCR and developed CMV colitis. At Day 42 post screening no response to therapy.
000053	Adenovirus	Continued Cidofovir and died of progressive adenovirus pneumonia
000063	Adenovirus	Continued Cidofovir and started IVIG. Blood levels resolved but persistent adenovirus in sputum on Day 42 for an evaluation of PR.
000066	CMV	Continued Cidofovir and Valcyte and then started Foscarnet. Developed CMV pneumonia and died.
000078	EBV	Received R-CHOP but died of progressive EBV and CMV infections
000082	CMV	Received Foscarnet and Chimerix CMV drug. Continued to have CMV viremia and died of GVHD and CMV.

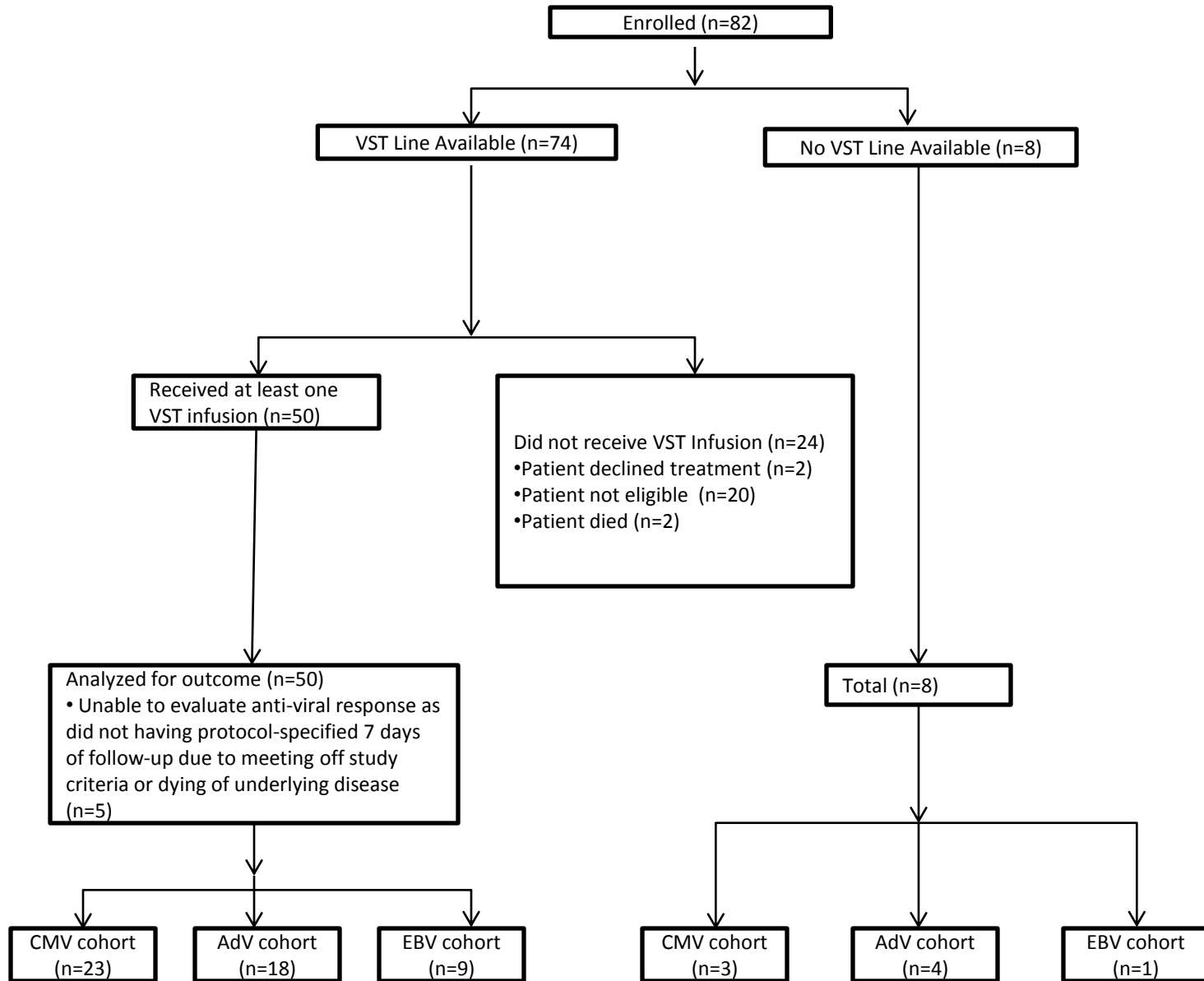
Supplementary Figure 1: Study Flow Diagram

SCCT CHALLAH TRIAL FLOW DIAGRAM

Enrollment

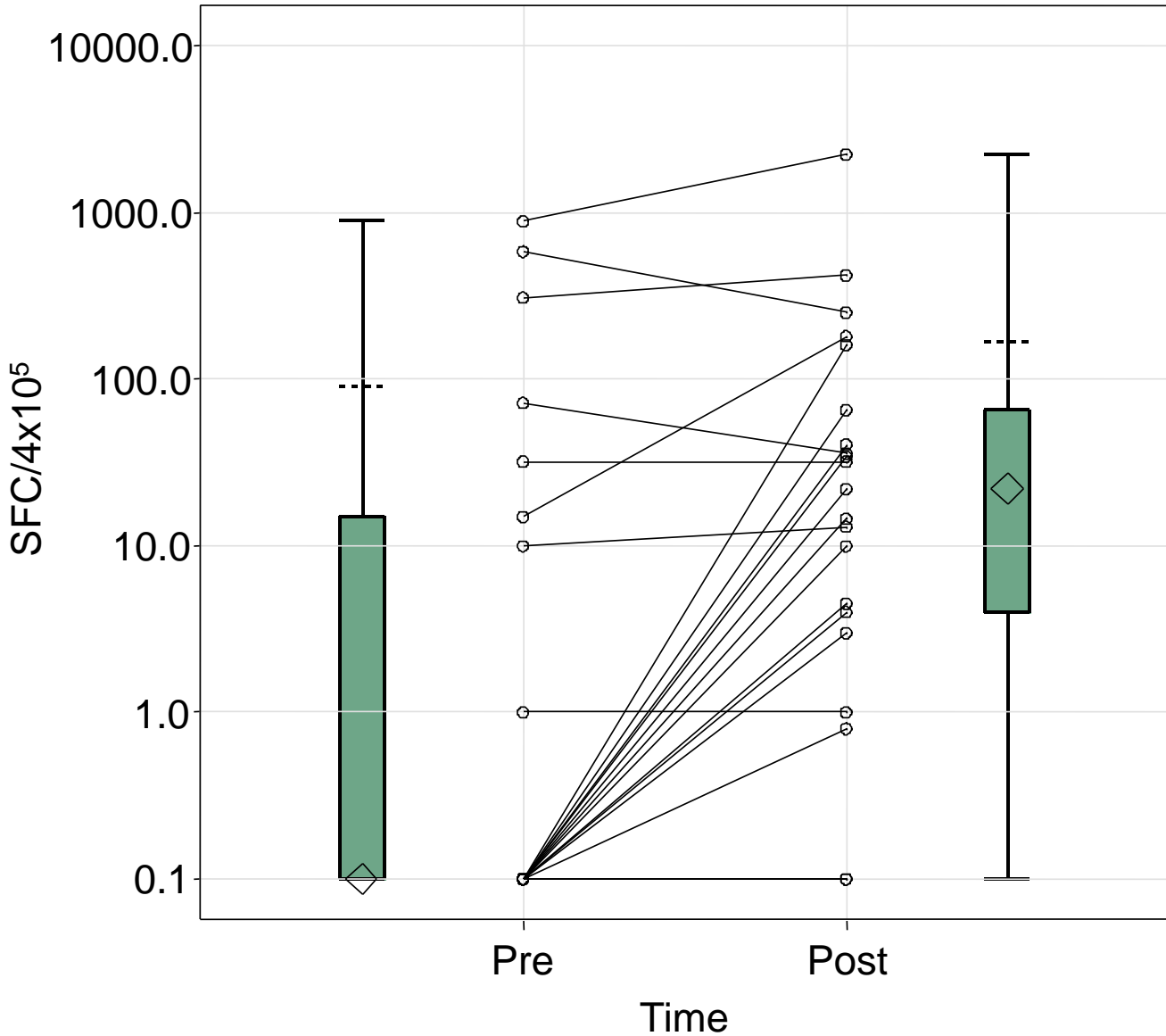
VST Line Search

Analysis



# Supplemental Fig. 2 – CMV immune reconstitution

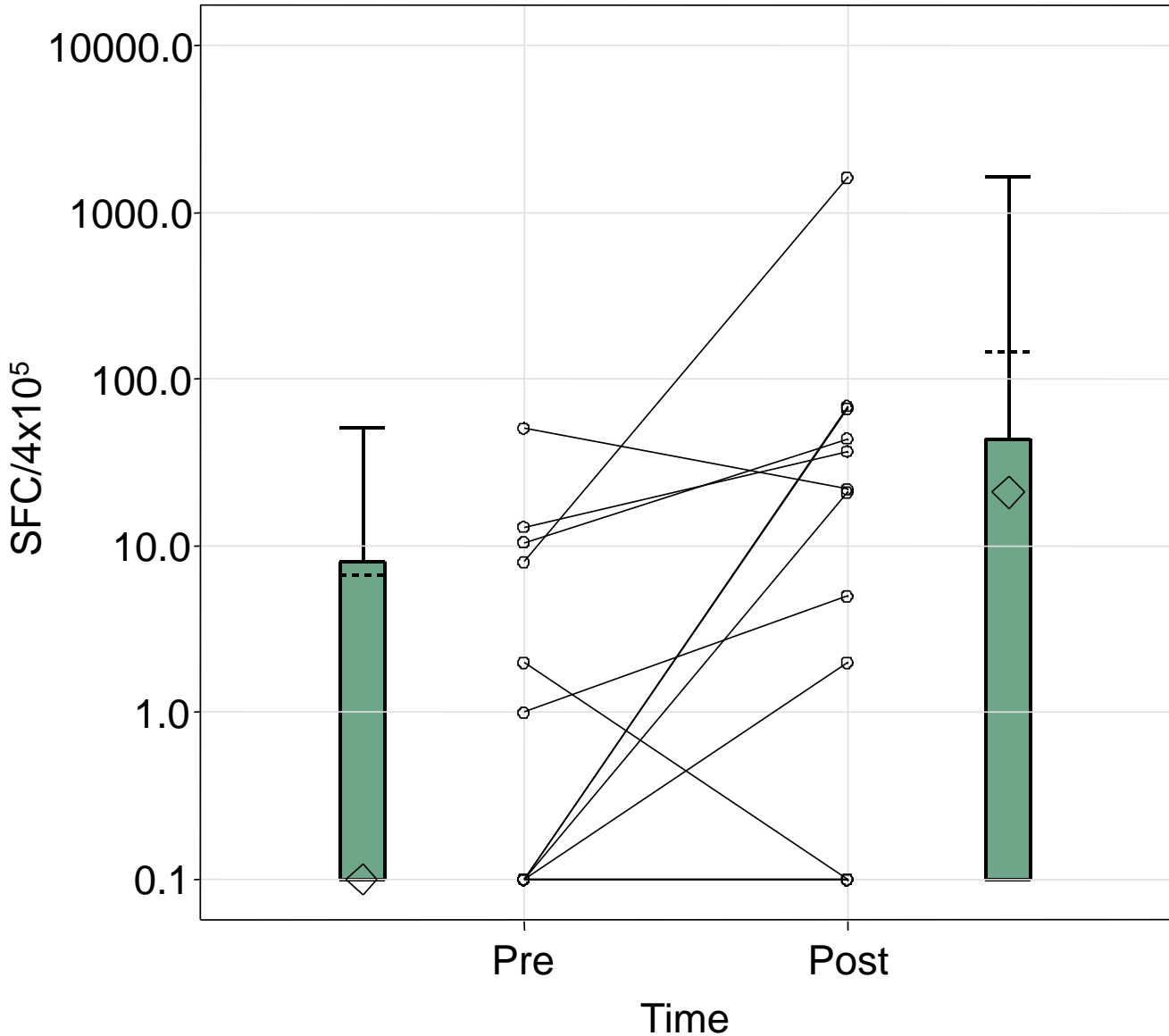
## Immune Reconstitution-CMV



*p=0.0150*

# Supplemental Fig. 3 – AdV immune reconstitution

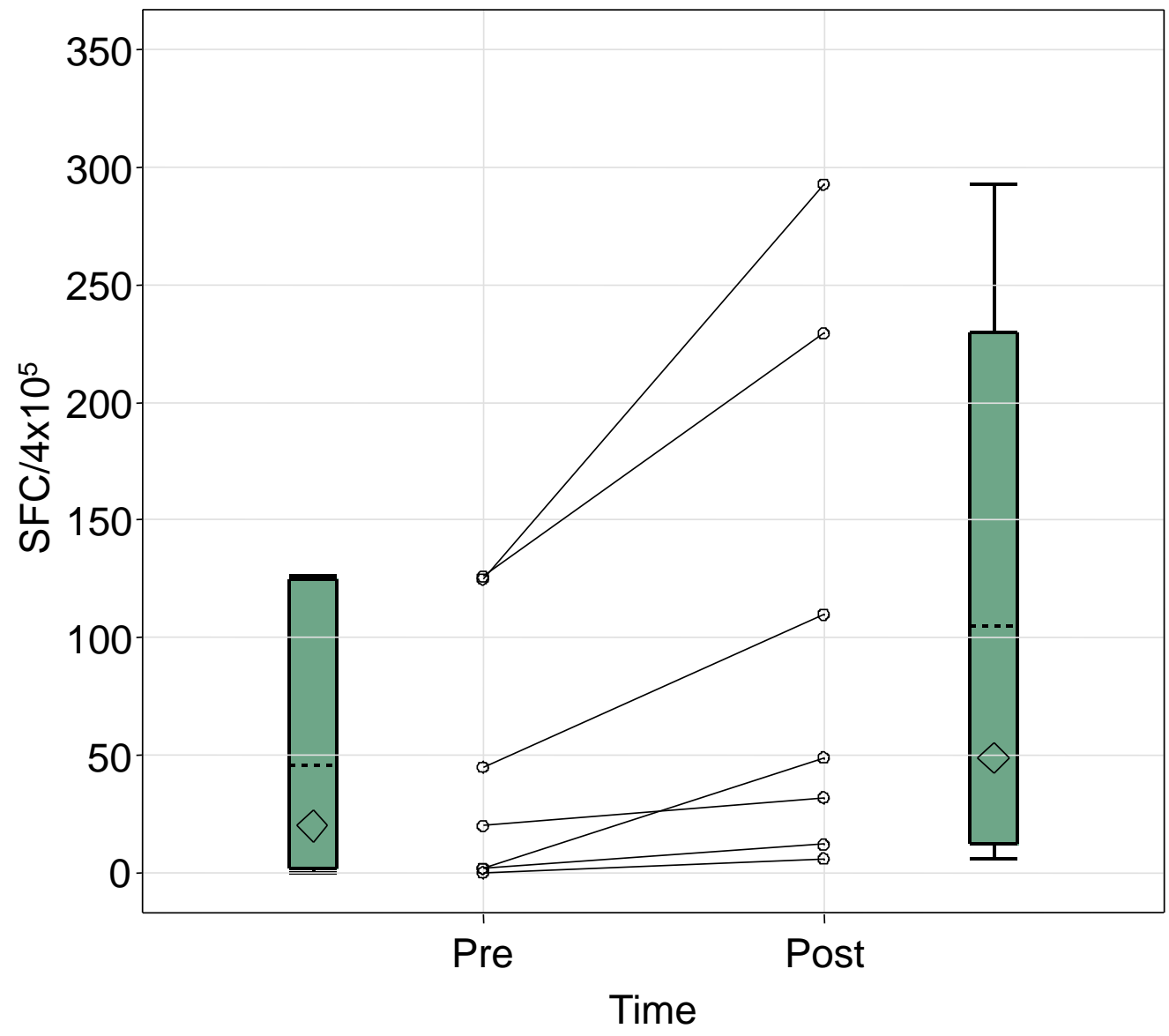
## Immune Reconstitution-Adenovirus



*p=0.0391*

# Supplemental Fig. 4 – EBV immune reconstitution

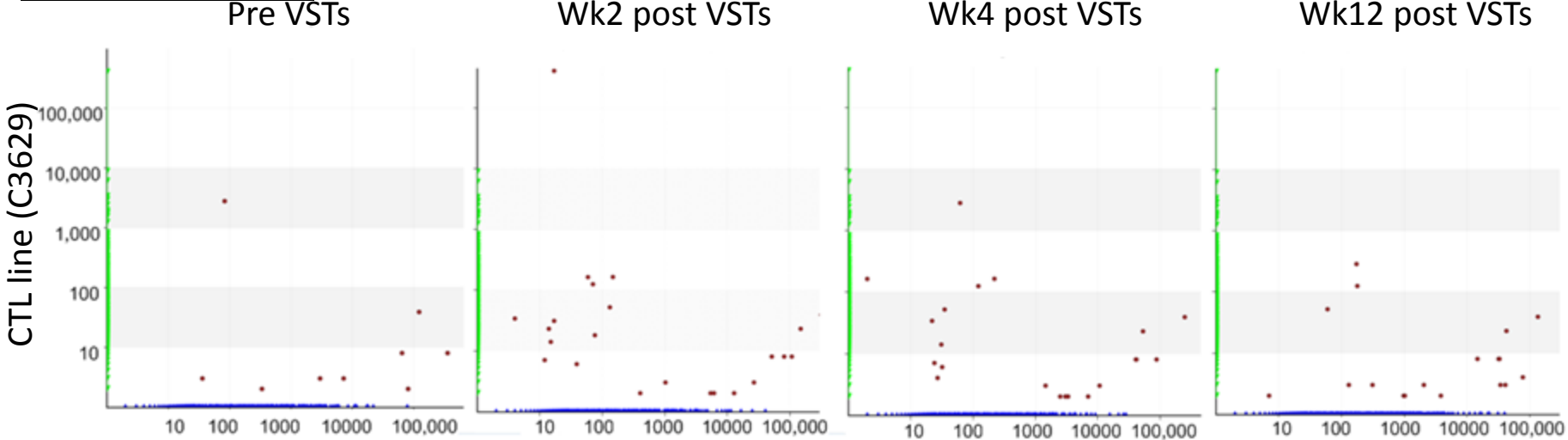
## Immune Reconstitution-EBV



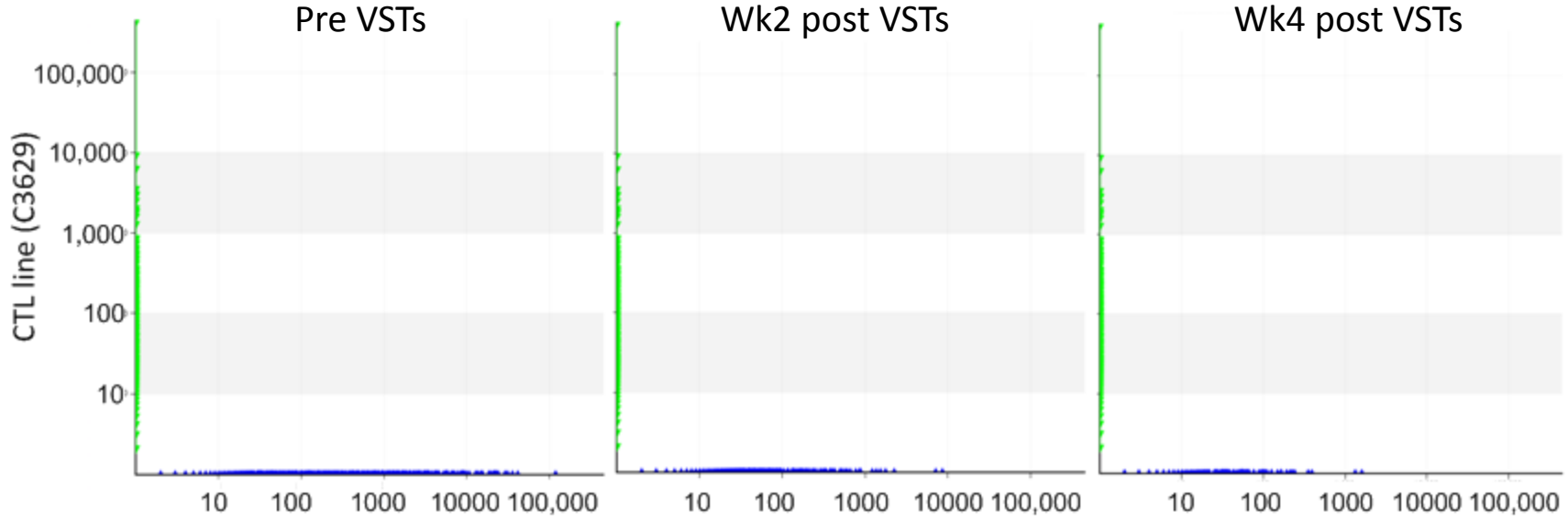
*p=0.0156*

# Supplementary Figure 5: Detection of VST -line derived V $\beta$ TCR sequences

## Pt69 - Responder



## Pt24 - Non-responder





## **LEGENDS FOR SUPPLEMENTARY FIGURES**

### **Supplementary Figure 1. Study Flow Chart**

Outline of the distribution of patients who were screened for participation in the study.

### **Supplementary Figure 2. CMV: Immune reconstitution**

Pre-treatment and peak frequencies of CMV-specific T-cells detected within 6 weeks after the first VST infusion, as measured by IFN $\gamma$  ELISpot assay following overnight stimulation of PBMCs with CMVpp65 pepmix. Results are expressed as SFC per  $4 \times 10^5$  input cells. The range of values is plotted adjacent to each line graph, together with the sign-rank P value. Dashed line, mean; diamond, median; upper whisker, maximum value; lower whisker, minimum value. The top and bottom of each box represent the 75% and 25% percentiles, respectively.

### **Supplementary Figure 3. AdV: Immune reconstitution**

Pre-treatment and peak frequencies of AdV-specific T-cells detected within 6 weeks after the first VST infusion, as measured by IFN $\gamma$  ELISpot assay following overnight stimulation of PBMCs with AdV Hexon and Penton pepmixes. Results are expressed as SFC per  $4 \times 10^5$  input cells. The range of values is plotted adjacent to each line graph together with sign rank P value. Dashed line, mean; diamond, median; upper whisker, maximum value; lower whisker, minimum value. The top and bottom of each box represent the 75% and 25% percentiles, respectively.

### **Supplementary Figure 4. EBV: Immune reconstitution**

Pre-treatment and peak frequencies of EBV-specific T-cells within 6 weeks after the first VST infusion as measured by IFN $\gamma$  ELISpot assay following overnight stimulation of PBMCs with EBV-LCLs. Results are expressed as SFC per  $4 \times 10^5$  input cells. The range of values is plotted adjacent to each line graph along with sign rank P values. Dashed line, mean; diamond,

median; upper whisker, maximum value; lower whisker, minimum value. The top and bottom of each box represent the 75% and 25% percentiles, respectively.

**Supplementary Figure 5. Detection of VST -line derived V $\beta$  TCR sequences in recipient blood**

Analysis of the T-cell repertoire in the infused line and peripheral blood of a responding patient (no. 69) before and after infusion using deep sequencing of TCR $\nu\beta$  CDR3 regions (top panel). Note that 11 sequences were shared between the infused line and peripheral blood before infusion, increasing to 23, 21, and 18 shared sequences detected at weeks 2, 4 and 6 post-infusion, respectively. By contrast, in a non-responding patient, there were no shared sequences detected either before or after VST infusion (bottom panel).

# **Most Closely HLA Matched Allogeneic Virus Specific Cytotoxic T-Lymphocytes (CTL) to Treat Persistent Reactivation or Infection with Adenovirus, CMV and EBV after Hemopoietic Stem Cell Transplantation (HSCT)**

## **SCCT PROTOCOL Version 0.8 (August 11, 2008)**

### **Study Chairperson**

Helen E Heslop, M.D. <sup>1</sup>

### **Protocol Team**

Joseph Antin, M.D.<sup>2</sup>  
David Avigan, M.D. <sup>8</sup>  
Karen K Ballen, M.D.<sup>3</sup>  
Malcolm K Brenner, M.D., Ph.D. <sup>1</sup>  
Catherine Bollard, M.D.<sup>1</sup>  
Richard Champlin, M.D.<sup>4</sup>  
Dennis Confer, M.D.<sup>5</sup>  
Adrian P Gee, Ph.D.<sup>1</sup>

Neena Kapoor M.D.<sup>9</sup>  
Donald Kohn, M.D.<sup>9</sup>  
Ann M Leen, Ph.D. <sup>1</sup>  
Adam Mendizabal, M.S. <sup>6</sup>  
Cliona M Rooney, Ph.D.<sup>1</sup>  
Elizabeth J Shpall, M.D.<sup>4</sup>  
Paul Szabolcs, M.D.<sup>7</sup>

### **Sponsored by the National Institutes of Health National Heart, Lung and Blood Institute**

- <sup>1</sup> Baylor College of Medicine
- <sup>2</sup> Dana Farber Cancer Institute
- <sup>3</sup> Massachusetts General Hospital
- <sup>4</sup> MD Anderson Cancer Center
- <sup>5</sup> National Marrow Donor Program
- <sup>6</sup> The EMMES Corporation
- <sup>7</sup> Duke University
- <sup>8</sup> Beth Israel Deaconess Medical Center
- <sup>9</sup> Children's Hospital of Los Angeles

### **Study Participants:**

Baylor College of Medicine  
The Methodist Hospital  
Texas Children's Hospital  
Beth Israel Deaconess Medical Center  
Children's Hospital of Los Angeles  
Dana Farber/Partners Cancer Center  
Duke University  
Massachusetts General Hospital  
University of Texas, MD Anderson Cancer Research Center

## PROTOCOL SYNOPSIS

### **Most closely HLA-Matched Allogeneic Virus Specific Cytotoxic T-Lymphocytes (CTL) to Treat Persistent Reactivation or Infection with Adenovirus, CMV and EBV after Hemopoietic Stem Cell Transplantation (HSCT)**

**Study Design:** The primary purpose of the study is to evaluate whether most closely HLA-matched multivirus specific CTL lines obtained from a bank of allogeneic viral specific cell lines have antiviral activity against three viruses: EBV, CMV and adenovirus.

Reconstitution of anti-viral immunity by donor-derived CTLs has shown promise in preventing and treating infections with CMV, EBV and adenovirus post-transplant. However, the time taken to prepare patient-specific products and lack of virus-specific memory T cells in cord blood and seronegative donors, limits their value. An alternative is to use banked most closely HLA-matched allogeneic CTLs. A Phase II study from Haque and colleagues showed activity when this approach was used to treat EBV+ve lymphomas arising after solid organ transplant.

In this trial, we will evaluate most closely HLA-matched multivirus specific CTLs (CHM-CTL) in transplant patients with CMV, adenovirus or EBV infection that is persistent despite standard therapy. The study agent will be assessed for safety (stopping rules defined) and antiviral activity.

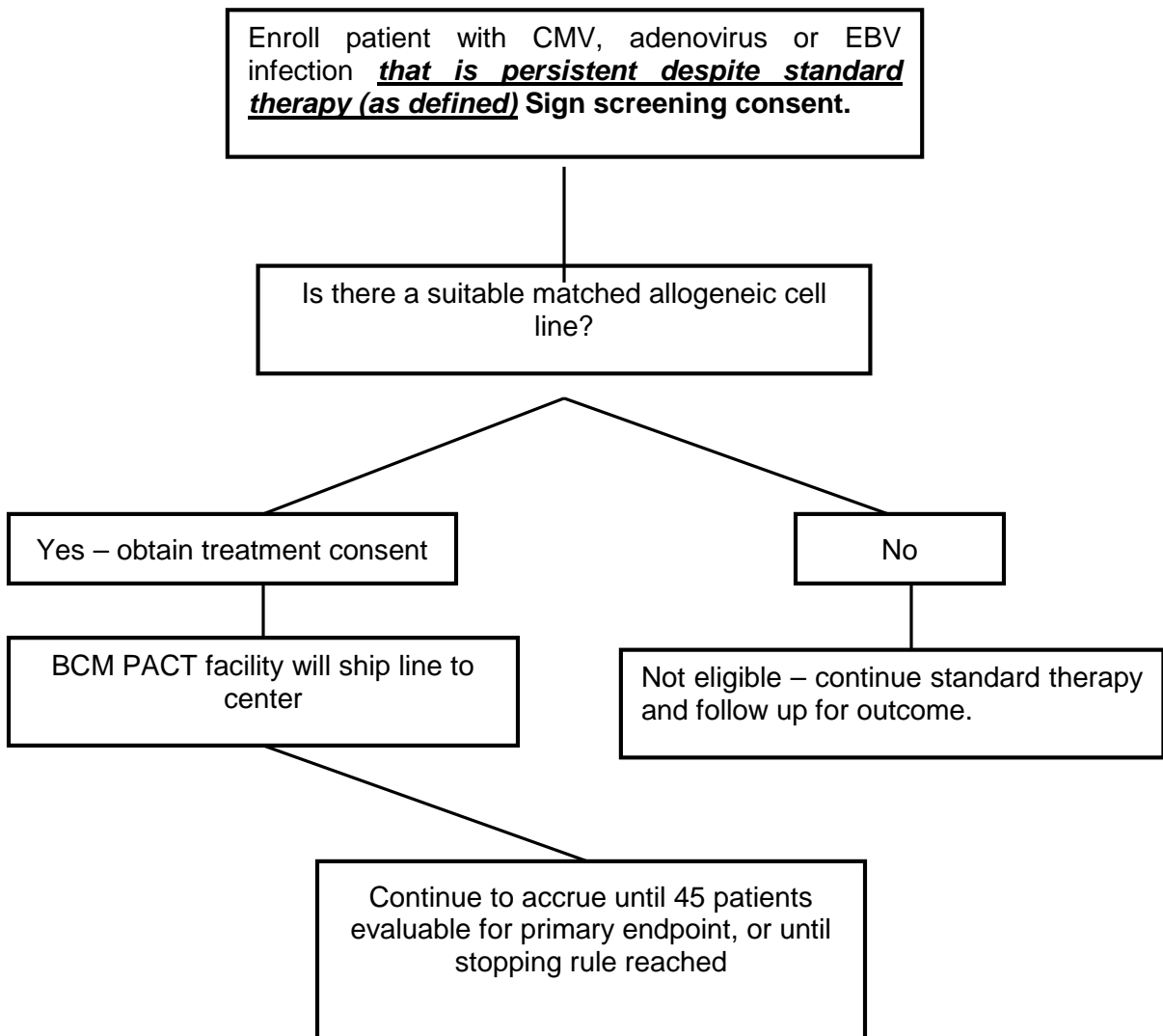
**Primary Objective:** The primary objective is to determine the feasibility and safety of administering CHM-CTLs to mediate antiviral activity in HSCT recipients with viral reactivation or infection. We will closely monitor the effects of these cells on GVHD.

**Secondary Objectives:** Secondary objectives are to determine: effects of CHM-CTL infusion on viral loads, reconstitution of antiviral immunity, persistence of infused CHM-CTLs and effects on clinical signs of viral infection. In addition, we will estimate the incidence of viral reactivations within 6 months. Additional endpoints include secondary graft failure, chronic GVHD, clinical response to CTL infusions, effects of HLA matching and overall survival.

**Eligibility:** Patients will be eligible following any type of allogeneic transplant if they have CMV, adenovirus or EBV infection persistent despite standard therapy (as defined). If patients are receiving steroids for treatment of GVHD or for other reasons, dosage must have been tapered to  $\leq 0.5$  mg/kg Prednisone (or equivalent) prior to study enrollment. Patients may not have received ATG, or Campath or other immunosuppressive monoclonal antibodies in the last 28 days. Patients must have an absolute neutrophil count (ANC) greater than  $500/\mu\text{L}$  with no active acute GVHD grades II-IV.

- Treatment Description:** The treatment schedule is as follows:  
Patients will receive  $2 \times 10^7$  CHM-CTL/m<sup>2</sup> as a single infusion. If they have a partial response (as defined) they are eligible to receive up to 4 additional doses at biweekly intervals. All patients should be followed with viral PCRs for the pertinent virus and will also have GVHD scores recorded at the intervals defined.
- Accrual Objective:** A maximum of 45 patients will be enrolled.
- Accrual Period:** The estimated accrual period is 24 months.
- Study Duration:** Patients will be followed for 12 months following initiation of therapy.

### STUDY DESIGN SCHEMATIC



## STUDY SCHEMA

**Aim:** To determine whether CHM-CTLs obtained from a bank of allogeneic viral specific cell lines are safe and have activity against three viruses EBV, CMV and adenovirus.

Inclusion Criteria	Exclusion Criteria
<ol style="list-style-type: none"><li>1. Prior allogeneic hematopoietic stem cell transplant using either bone marrow, PBSC or cord blood within one year.</li><li>2. CMV, adenovirus or EBV infection persistent despite standard therapy (see 2.3 for definitions).</li><li>3. Clinical status at enrollment to allow tapering of steroids to not more than 0.5 mg/kg/day prednisone.</li><li>4. Signed informed consent and/or assent line.</li><li>5. Educational materials provided to, and reviewed with, patients under the age of 18.</li><li>6. Negative pregnancy test in female patients of childbearing potential who have received a reduced intensity conditioning regimen.</li></ol>	<ol style="list-style-type: none"><li>1. Adults unable to provide informed consent</li><li>2. Unable to wean steroids to not more than 0.5 mg/kg/day prednisone.</li><li>3. Received ATG, or Campath or other T cell immunosuppressive monoclonal antibodies in the last 28 days.</li><li>4. Patients with other uncontrolled infections (see 2.3.2 for definitions)</li><li>5. No active acute GVHD grades II-IV.</li><li>6. Received donor lymphocyte infusion in last 28 days</li><li>7. Active and uncontrolled relapse of malignancy</li></ol>

If any improvement after 1 dose in the absence of toxicity, up to 4 additional doses at biweekly intervals will be given.

### **Primary endpoint:**

Feasibility of finding suitable line

Safety including acute GvHD by day 45 and infusion-related adverse events or nonhematological adverse events within 30 days of the last CTL infusion

### **Secondary endpoints:**

- Antiviral responses at Day 28
- Effects of CHM-CTL on viral loads
- Reconstitution of antiviral immunity at 2 weeks, 1 and 3 months
- Persistence of infused CHM-CTLs and effects on clinical signs of viral infection
- Viral reactivations within 6 months
- Secondary graft failure at 30 days
- Chronic GVHD at 6 and 12 months
- Clinical response to CTL infusions at 6 weeks and 3 months
- Effects of HLA matching
- Overall Survival at 6 and 12 months

If the patient progresses within 14 days or no response within 28 days, then treat with alternative therapy off study, but follow for study endpoints.

## TABLE OF CONTENTS

<b>1</b>	<b>BACKGROUND AND RATIONALE</b>	<b>7</b>
1.1	Viral Infection Post Transplant	7
1.2	CMV	7
1.3	EBV	7
1.4	Adenovirus	8
1.5	Adoptive Immunotherapy with Virus Specific CTLs	8
1.6	Rapid Generation Techniques and Selection	10
1.7	Most closely Matched Allogeneic Lines	10
1.8	Risks of Administering Donor CTLs	11
<b>2</b>	<b>STUDY DESIGN</b>	<b>13</b>
2.1	Study Design	13
2.2	Primary Objective and Rationale for Study Design	13
2.3	Eligibility	13
2.4	Treatment Plan	15
<b>3</b>	<b>STUDY ENDPOINTS</b>	<b>20</b>
3.1	Primary Endpoints	20
3.2	Secondary Endpoints	21
<b>4</b>	<b>PATIENT ENROLLMENT AND EVALUATION</b>	<b>22</b>
4.1	Enrollment	22
4.2	Study Monitoring	22
<b>5</b>	<b>STATISTICAL CONSIDERATIONS</b>	<b>32</b>
5.1	Study Design Synopsis	32
5.2	Accrual	32
5.3	Primary Endpoint	32
5.4	Sample Size and Power Calculations	32
5.5	Stopping Guidelines	34
5.6	Demographic and Baseline Characteristics	36
5.7	Endpoints and Analysis Plan	36
5.8	Data and Safety Monitoring Board (DSMB)	36

## LIST OF APPENDICES

### APPENDIX A REFERENCES

### APPENDIX B INFORMED CONSENTS AND EDUCATIONAL MATERIAL



## CHAPTER 1

### 1 BACKGROUND AND RATIONALE

#### 1.1 Viral Infection Post Transplant

During the period of immune recovery after hematopoietic stem cell transplantation (HSCT) viral infections, which are normally controlled by T-cell immunity are an important cause of morbidity and mortality. The degree of risk for infection is dictated by the degree of tissue mismatch between donor and recipient, and the resultant degree of immunosuppression as well as the immune status of the donor. Reactivation of latent viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex and herpes zoster are common and often cause symptomatic disease. Respiratory viruses such as adenovirus, influenza, and respiratory syncytial virus (RSV) also frequently cause infection. Antiviral pharmacologic agents are effective against only some of these viruses; their use is costly, associated with significant toxicities and the outgrowth of drug-resistant mutants. As delay in recovery of virus-specific cellular immune response is clearly associated with viral reactivation and disease in these patients, cellular immunotherapy to restore viral-specific immunity is an attractive option that has been applied to target three common viruses; CMV, EBV and adenovirus.

#### 1.2 CMV

Cytomegalovirus (CMV) is a lytic virus that usually causes an asymptomatic infection in immunocompetent individuals. It persists in approximately 70% of healthy adults and replicates in epithelial cells, fibroblasts and monocytes. Reactivation of CMV in the stem cell recipient can result in significant morbidity and mortality, with clinical manifestations including interstitial pneumonitis, gastroenteritis, fevers, hepatitis, encephalitis and retinitis.<sup>1</sup> Cell-mediated immunity is considered the most important factor in controlling CMV infection and CMV-specific CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes play an important role in immune protection both primary infection and subsequent reactivations. The most frequently used drugs for prophylactic or preemptive therapy are ganciclovir and foscarnet. These drugs have been successful in reducing mortality associated with CMV disease and in preventing early CMV disease in combination with intravenous immune globulin. However, both have significant side effects including neutropenia and nephrotoxicity.

#### 1.3 Epstein-Barr Virus

Epstein-Barr virus (EBV) is a gammaherpesvirus that infects more than 95% of the world's population. Primary infection usually produces a mild self-limiting disease, which is followed by latent infection in B cells and productive replication in B cells and mucosal epithelium. There are at least four types of viral latency: type 1, expressing only the virus nuclear antigen 1 (EBNA1); type 2, expressing in addition to EBNA1, the latent membrane proteins, LMP1 and LMP2; and type 3, expressing all the seven latency-associated proteins including the immunodominant EBNA3 viral antigens.<sup>2</sup> In these types of latency the viral small RNAs and EBERs are abundantly expressed as well as transcripts from the BamHI A region of the viral genome, BARTs. In the fourth type of latency, found in the majority of circulating B cells of healthy individuals, no viral RNA expression can be detected. In immunocompromised hosts, outgrowth of B cells expressing Type 3 latency, which are highly susceptible to virus specific T

cells, may lead to the development of post-transplant lymphoproliferative disease (PTLD). The overall incidence of PTLD after HSCT is less than 1%, but the incidence is increased in recipients with an underlying diagnosis of immunodeficiency and for recipients of stem cells from unrelated or human-leukocyte-antigen (HLA)-mismatched donors who receive grafts that are selectively depleted of T cells to prevent graft-versus-host disease (GVHD).<sup>3-5</sup>

Few small molecule drugs have any effect on B cells already transformed by EBV, although nucleoside analogs, like ganciclovir, do inhibit its replicative cycle. Chemotherapy is rarely effective and associated with significant toxicity. One option for prophylaxis and treatment of PTLD after HSCT is rituximab, a monoclonal antibody against the B cell phenotypic antigen, CD20. Response rates to rituximab between 55% and 100% have been reported in different series.<sup>5-7</sup> However, not all patients respond and rituximab produces depletion of normal B-cell for more than 6 months. This can be problematic in a patient population that is already immunosuppressed.

#### **1.4 Adenovirus**

Adenovirus is a nonenveloped lytic DNA virus and humans are susceptible to infection with 51 serotypes of adenovirus, forming six distinct species (A to F), which differ in their tissue specificity and virulence. Although acutely infecting viruses, adenoviruses may persist for many months after resolution of disease and therefore are frequently carried undetected into the transplant by donor or recipient. Acute infection is rarely fatal in healthy adults; it is significant cause of morbidity and mortality in immunocompromised individuals, in whom it may produce pneumonia, hemorrhagic cystitis, nephritis, colitis, hepatitis, and encephalitis. Adenovirus has a particularly high incidence after pediatric HSCT.<sup>8</sup> Several reports have shown that clearance of adenovirus infection is associated with detection of adenovirus specific T cells<sup>9;10</sup> and recovery is significantly delayed in recipients of matched unrelated donor and haploidentical transplant who receive intensive immunosuppression such as Campath.<sup>10</sup> The most frequently used drug for disease treatment is Cidofovir, but the associated nephrotoxicity is a major concern and in the absence of prospective, randomized, controlled trials, the efficacy of the drug is uncertain.

#### **1.5 Adoptive Immunotherapy with Virus Specific CTLs**

Since recovery of virus-specific T cells is clearly associated with protection from infection with each of these viruses,<sup>9;11;12</sup> adoptive immunotherapy to decrease the time to immune reconstitution is an attractive approach. Virus-specific T cells generated by repeated stimulation with antigen presenting cells expressing viral antigens have been evaluated in clinical trials to prevent and treat viral infections in immunocompromised hosts. This approach eliminates alloreactive T cells.

There are several considerations in developing protocols for generating virus-specific CTLs *ex vivo*. Knowledge of the immunodominant antigens that induce protective T cells specific for the targeted virus is required and a delivery system to transfer the antigen to effective antigen-presenting cells (APCs) must be identified. The APC must be autologous, express major histocompatibility complex (MHC) antigens presenting relevant virus-derived peptides as well as co-stimulatory molecules sufficient to induce T cell activation and expansion. These reagents all need to be suitable for GMP manufacturing which limits the use of some types and sources of antigen.

### 1.5.1 T Cell Therapy for CMV

The first study evaluating whether adoptively-transferred T cells could reconstitute anti-viral immunity targeted CMV. In this study, CMV-specific T-cell clones were derived from sibling donors after stimulation with autologous fibroblasts pulsed with CMV.<sup>13</sup> There were no adverse effects and CMV-specific immune responses were reconstituted, with none of the patients developing CMV disease or late recurrence. Anti-CMV activity did decline, however, in recipients who did not develop CD4<sup>+</sup> CMV-specific T-cell responses, suggesting CD4 T cells may be necessary for long-term persistence. In another prophylaxis study, Peggs et al., generated CMV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells by stimulation of peripheral blood mononuclear cells (PBMC) with dendritic cells pulsed with CMV antigens derived from a CMV-infected human lung fibroblast cell line<sup>14</sup>. Small doses of CTLs were able to reconstitute immunity with considerable in-vivo expansion of CMV-specific CTLs. To avoid the use of live CMV during T cell manufacture, a more recent study stimulated CTLs with dendritic cells pulsed with the HLA-A2 restricted peptide NLV derived from the cytomegalovirus-pp65 protein.<sup>15</sup> While this approach also appeared effective, a concern is the restricted specificity of the infused CTLs, since targeting a single epitope may allow escape variants and limits the study to patients who are HLA A2-positive.

CMV-specific CTLs have also been used therapeutically in patients with CMV infection that has persisted or recurred despite prolonged antiviral medication.<sup>16</sup> The results were encouraging with suppression of viral reactivation in 6 of 7. In this study, the source of antigen was a CMV lysate, which has the advantage of producing a broad immune response but which is not suitable for Phase III studies because of the risk of infection from live virus in the lysate.

The CTL therapies described employed methods of T cell production that required prolonged periods of activation and expansion and also require specialized GMP facilities and significant regulatory support. This reduces the practicality of adoptive immunotherapy, since CTL lines must be made long in advance of disease, and few centers have the facilities or infrastructure required for this type of cell processing. A recently reported clinical trial using tetramer selection of CMV peptide-specific T cells directly from peripheral blood avoided both ex vivo expansion and live viral antigens. Although exclusively CD8<sup>+</sup> T cells were infused, they expanded by several logs after infusion, clearing infection in 8/9 cases.<sup>17</sup> Again this approach is limited by the availability of tetramers for uncommon HLA types and lack of class II tetramers.

### 1.5.2 T Cell Therapy for EBV

The outgrowing EBV-infected B cells of PTLD have the same phenotype and viral antigen expression as the EBV-transformed lymphoblastoid cell lines (LCLs) generated by infecting peripheral blood B cells with a laboratory strain of EBV. As LCLs can be readily prepared from any donor, they have been used as antigen presenting cells in clinical studies evaluating EBV specific CTLs.<sup>7,18-21</sup> EBV-specific CTLs generated using this methodology are polyclonal, contain both CD4<sup>-</sup> and CD8<sup>-</sup> positive EBV-specific T cells, and recognize multiple latent and lytic viral antigens. We have shown that adoptively transferred EBV-specific CTLs can survive for up to 8 years after infusion, expand up to 2-4 logs after infusion, and reduce the high virus load that is observed in about 20% of patients.<sup>18,20</sup> In a study targeting a high-risk patient population receiving T cell-depleted marrow, none of 59 patients who received EBV CTLs as prophylaxis developed PTLD.<sup>12</sup> Of six patients with active PTLD at the time of infusion, donor derived EBV-specific CTL lines induced remission in five, while in the sixth the tumor virus had

deleted the immunodominant epitopes in EBNA3 that were the targets of the infused effector T cells.<sup>22</sup> Other studies have confirmed the activity of EBV specific CTLs post transplant.<sup>7;21</sup>

### 1.5.3 T Cell Therapy for Adenovirus.

Feuchtinger et al. treated patients with adenovirus infection using CD4+ and CD8+ adenovirus-specific T cells isolated from the donor after a short in vitro stimulation with adenovirus viral antigen followed by selection of gamma-interferon-secreting cells.<sup>23</sup> Small numbers of adenovirus-specific donor T cells were infused into nine children with systemic adenovirus infection after HSCT. Adenovirus specific immune responses were detected in five of six evaluable patients, associated with a sustained decrease in viral load and clearance of infection.<sup>23</sup>

### 1.5.4 Multivirus-Specific T Cells

The strategies described above each only target one virus. To broaden the specificity of single CTL lines to include the three most common viral pathogens of stem cell recipients, we reactivated CMV and adenovirus-specific T cells by using mononuclear cells transduced with a recombinant adenoviral vector encoding the CMV antigen pp65. Subsequent stimulations with EBV-LCL transduced with the same vector both reactivated EBV-specific T cells and maintained the expansion of the activated adenovirus and CMV-specific T cells.<sup>24</sup> This method reliably produced CTLs with cytotoxic function specific for all three viruses, which we infused into 14 stem cell recipients in a Phase I prophylaxis study. We observed recovery of immunity to CMV and EBV in all patients but an increase in adenovirus-specific T cells was only seen in patients who had evidence of adenovirus infection pre-infusion.<sup>24</sup> All patients with pre-infusion CMV, adenovirus or EBV infection or reactivation were able to clear the infection, including one patient with severe adenoviral pneumonia requiring ventilatory support.<sup>24</sup> CTLs recognizing multiple antigens can therefore produce clinically relevant effects against all three viruses.

## 1.6 Rapid Generation Techniques and Selection

Peptide-HLA tetramers and cytokine-secretion capture columns allow rapid isolation of antigen-specific T cells for adoptive transfer. With tetramer selection, T cells that bind a particular peptide can be isolated magnetically for direct transfer to patients without the need for a prolonged in vitro culture. This approach requires knowledge of immunodominant epitope and is restricted by HLA type, and although it successfully selects CMV specific cells, which are abundant in peripheral blood, it is of more limited value when trying to isolate T cells with specificities that circulate at low frequency.<sup>17</sup> . In addition production of clinical-grade tetramers for multiple HLA types for use under GMP may limit wider application of this strategy. Capture of T cells that secrete cytokines in response to stimulation with viral antigens also allows rapid T cell selection as demonstrated for adenovirus specific T cells.<sup>23</sup> This strategy has the advantage that knowledge of epitopes is not required and there is no HLA restriction. Unfortunately it is inefficient, producing small numbers of selected cells impeding the product characterization essential to later phase clinical study.

## 1.7 Most closely Matched Allogeneic Lines

An alternative approach that bypasses the need to grow CTLs for individual patients is to bank closely HLA-matched allogeneic cytotoxic T lymphocyte lines (CHM-CTLs) that could be

available as an “off the shelf” product. A concern with this approach is that the in vivo persistence of a mismatched product may be suboptimal after administration, as the recipient may generate an immune response to the non-shared HLA antigens. However, a number of small studies showed the feasibility of this approach and reported clinical responses in the patients with EBV lymphoma arising after HSCT or solid organ transplant.<sup>25;26</sup> More recently a Phase II study using EBV-specific CTLs to treat PTLD after solid organ transplant or SCT has shown an encouraging response rate of 64% and 52% at 5 weeks and 6 months, respectively, with higher response rates observed at six months in patients who received the most closely HLA-matched CTL lines.<sup>27</sup> In this study patients received 4 doses of  $2 \times 10^6$  CTL/kg at weekly intervals. Lines were selected for matching by low resolution typing and screened for high level killing of autologous LCLs and low level killing of patient PHA blasts. The degree of HLA matching ranged from 2/6 to 5/6 antigens and there was a statistically significant trend towards a better outcome at 6 months with better matching. Importantly no patient developed GVHD post CTL administration.<sup>27</sup> In another report two solid organ recipients with CNS lymphoma received closely matched EBV-specific T cells resulting in complete resolution of their brain lesions.<sup>28</sup>

This approach, therefore, warrants further evaluation. In this protocol we propose to evaluate if using most closely matched allogeneic CTLs is a safe and feasible strategy for treating patients post-HSCT with CMV, adenovirus or EBV infection persistent despite standard therapy.

### **1.8 Risks of Administering Donor CTLs**

Over 65 patients have been treated on our previous adoptive immunotherapy protocol in which allogeneic donor derived EBV-specific CTL were administered after bone marrow transplantation. The only significant complication was an inflammatory response in a patient with bulky EBV lymphoma. In addition 18 patients have received trivirus specific CTLs and 12 patients received adenovirus and EBV specific CTLs without developing significant adverse events.

None of the patients treated on any of these studies developed significant de novo GVHD. In other reported studies none of the patients treated with CMV CTL by Walter developed GVHD. In the cohort of patients treated by MacKinnon et al., 3/13 patients developed mild (Grade I) GVHD; since immunosuppression had been withdrawn early in this study, it is unclear if this side effect was due to CTL infusion.<sup>29</sup> Although there is theoretically an increased risk of GVHD due to greater mismatch with partially matched CTLs, no GVHD was reported in the Phase II study of closely matched allogeneic CTLs or in several case reports using matched CTLs.<sup>26;27</sup>

In our studies with donor-derived CTLs we have screened CTL lines for reactivity against other host tissues such as fibroblasts and/or PHA blasts as a release criteria. However, there is no completely reliable in vitro assay for excluding this possibility and performing such an assay in the current protocol would be difficult for two reasons. First, many recipients would not have residual pre-transplant lymphocytes available to make PHA blasts and their blood at the time of study eligibility determination would be donor and therefore not a valid predictive target. Second, it would add 10 days to the release time which would adversely affect feasibility and perhaps outcome. We do not therefore propose this assay as a release criteria. We will, however, take several additional precautions to minimize the risk of GVHD. First, any patient with preexisting GVHD of > Grade 2 will be excluded from the study. Second, we will administer CTLs at a dose of  $2 \times 10^7$  cells/m<sup>2</sup>. This is a much smaller number of T cells than administered at the time of an unmanipulated marrow infusion.

Another potential hazard is the infusion of EBV transformed B cells, which have been cocultured with the CTL during generation of the T cell lines. This is unlikely to constitute an additional risk to the recipient for several reasons. First, the lymphoblastoid cells are not viable because they have been irradiated with 4000cGy and cocultured with known effectors. In addition, we will add acyclovir to the LCL cultures so no productive virus will be present in cultures. Finally we will monitor levels of EBV DNA in peripheral blood by PCR before and after CTL infusions. The laboratory strain of EBV used for LCL production has not been detected in over 200 patients who have received EBV-specific CTLs so far. In addition, the CTL lines are phenotyped prior to infusion and all lines have to meet release criteria (<2% CD19+ve B cells in the CTL product for infusion.)

Infusion of cells infected with the adenoviral vector (Ad5f35pp65), could theoretically lead to an inflammatory response due to infectious virus or viral antigens. This is unlikely for several reasons. First, the adenoviral vector is RCA negative. Secondly, in this study we are initially using the adenoviral vector to infect peripheral blood mononuclear cells to stimulate the T cells. We have shown that only CD14+ve cells (monocytes) and not T cells or B cells become infected with the adenoviral vector at the MOI we use. Subsequent stimulations of the T cells will be with lethally irradiated monocytes and then with EBV-LCL lines transduced with the Ad5f35 vector. Prior to stimulation, the irradiated LCL and monocytes will be washed 4 times which we have shown eliminates 4 logs of free adenovirus from the cultures. Thirdly, Ad-specific CTL will be cultured for at least 7 days after the last stimulation with LCL infected with the Ad5f35 vector since pre-clinical studies have shown that this ensures no Ad-infected LCL remain alive to be administered to the patients. Finally, we will not infuse any T cells which contain >2% CD19 positive cells (B cells) or >2% CD14 positive cells (monocytes).

## CHAPTER 2

### 2 STUDY DESIGN

#### 2.1 Study Design

This trial is designed to evaluate the feasibility, safety and efficacy of most closely HLA-matched multivirus specific CTL lines (CHM-CTLs) in HSCT patients with EBV, CMV or adenovirus infections that are persistent despite standard therapy.

#### 2.2 Primary Objective and Rationale for Study Design

The primary purpose of the study is to assess the safety of administering CHM-CTLs in transplant patients with EBV, CMV, or adenovirus infection. We have elected to use a dose of  $2 \times 10^7$  CHM-CTLs/m<sup>2</sup> that has been shown to be safe and have clinical activity in a Phase I study using donor specific CTLs. Because the persistence of adoptively transferred cells may be shorter using banked most closely matched lines, we will give the option of administering additional doses in subjects that have a partial response after one dose.

It is unclear what degree of matching will be needed for clinical activity so we propose to allow infusion of lines that match at only one antigen although preference will be given to lines matching at the most loci.

For CMV infection, standard therapy is well defined as antiviral agents with Ganciclovir being the agent of choice and Foscarnet or Cidofovir being effective second line agents. For EBV infection, rituximab is the current treatment of choice for patients with CD20+ lymphoma. For patients with CD20- tumors there is no clear standard of therapy although most physicians would likely administer chemotherapy. There is also no clear standard therapy for adenovirus although there are reports of Cidofovir having activity. We have therefore chosen to use Cidofovir as standard therapy although this requirement would be waived if the subject could not tolerate this agent due to nephrotoxicity.

#### 2.3 Eligibility

##### 2.3.1 Inclusion Criteria

Patients will be eligible following any type of allogeneic transplant if they have CMV, adenovirus or EBV infection persistent to standard therapy (as defined below).

1. Prior allogeneic hematopoietic stem cell transplant using either bone marrow, peripheral blood stem cells or cord blood within 1 year. Recipients of non-myeloablative transplants are also eligible.
2. CMV, adenovirus or EBV infection persistent despite standard therapy
  - a. For CMV infection<sup>30;31</sup>
    - i. Patients with CMV disease: defined as the demonstration of CMV by biopsy specimen from visceral sites (by culture or histology) or the

detection of CMV by culture or direct fluorescent antibody stain in bronchoalveolar lavage fluid in the presence of new or changing pulmonary infiltrates OR

- ii. Failure of antiviral therapy: defined as the continued presence of pp65 antigenemia (>1+ cell/100,000 cells) or DNAemia (as defined by reference lab performing PCR assay but usually >400 copies/ml) after at least 7 days of antiviral therapy OR
- iii. Relapse after antiviral therapy defined as recurrence of either pp65 antigenemia or DNAemia after at least 2 weeks of antiviral therapy
- iv. For CMV infection, standard therapy is defined as 7 days therapy with Ganciclovir, Foscarnet or Cidofovir for patients with disease (see 2.a.i) or recurrence after 14 days therapy (see 2.a.iii)

b. For EBV infection

- i. EBV infection is defined as
  1. Biopsy proven lymphoma with EBV genomes detected in tumor cells by immunocytochemistry or in situ PCR
  2. Or clinical or imaging findings consistent with EBV lymphoma and elevated EBV viral load in peripheral blood.
- ii. For EBV infection, standard therapy is defined as rituximab given at 375mg/m<sup>2</sup> in patients with a CD20+ve tumor
- iii. Failure is defined as
  1. There was an increase or less than 50% response at sites of disease for EBV lymphoma
  2. There was a rise or a fall of less than 50% in EBV viral load in peripheral blood or any site of disease

c. For adenovirus infection

- i. Adenovirus infection is defined as the presence of adenoviral positivity as detected by PCR, DAA or culture from ONE site such as stool or blood or urine or nasopharynx.
- ii. Adenovirus disease will be defined as the presence of adenoviral positivity as detected by culture from more than two sites such as stool or blood or urine or nasopharynx
- iii. Standard therapy is defined as 7 days therapy with Cidofovir (if renal function permits this agent to be given)
- iv. Failure is defined as a rise or a fall of less than 50% in viral load in peripheral blood or any site of disease as measured by PCR or any other quantitative assay)

3. Clinical status at enrollment to allow tapering of steroids to less than **0.5 mg/kg/day** prednisone.
4. Written informed consent and/or signed assent line from patient, parent or guardian.
5. Education materials have been provided to, and reviewed with, patients under the age of 18.



- 6 Negative pregnancy test in female patients of childbearing potential who have received a reduced intensity conditioning regimen.

### 2.3.2 Exclusion Criteria

1. Patients receiving ATG, or Campath or other immunosuppressive T cell monoclonal antibodies within 28 days of screening for enrollment.
2. Patients with other uncontrolled infections. For bacterial infections, patients must be receiving definitive therapy and have no signs of progressing infection for 72 hours prior to enrollment. For fungal infections patients must be receiving definitive systemic anti-fungal therapy and have no signs of progressing infection for 1 week prior to enrollment.

Progressing infection is defined as hemodynamic instability attributable to sepsis or new symptoms, worsening physical signs or radiographic findings attributable to infection. Persisting fever without other signs or symptoms will not be interpreted as progressing infection.

3. Patients who have received DLI within 28 days.
4. Patients with active acute GVHD grades II-IV.
5. Active and uncontrolled relapse of malignancy

### 2.3.3 Informed Consent

The informed consent process will begin at recognition of subject eligibility and consent will be obtained per institutional practices before study therapy is initiated. Subjects will initially sign a screening consent to enable a search to be made for a line. If a line is available they will sign the treatment consent.

### 2.3.4 Donor Eligibility

Donors for these CTLs lines fall into several categories

- 2.3.4.1 Some CTL lines were previously manufactured for our IRB approved protocol H12683. These donors were initially chosen as transplant donors because they were the best match with for the original transplant recipient and they met eligibility criteria. They were subsequently consented for enrollment on H12683 to provide blood for generation of trivirus-specific CTL lines. There are either additional vials available after the product was used for the original recipient or the original recipient was ineligible. These donors gave consent for lines not used for the original recipient to be used for research.
- 2.3.4.2 We will manufacture additional lines for specific use for this study from individuals with common HLA types. Some of these individuals will be subjects who have previously enrolled on our IRB approved research study (H7634) to manufacture CTL lines from normal donors for preclinical studies so that we know that their lines have broad antiviral reactivity. We will approach these donors previously enrolled on this study to ascertain if they are willing to enroll and donate blood for the current study. We will also collaborate

with the National Marrow Donor Program which is a registry of HLA-typed donors who have volunteered for transplant donation to obtain additional donors after the protocol is approved by the NMDP IRB.

For these newly manufactured lines, donors will be evaluated by either Dr Carrum who is director of the donor center at BCM or an NMDP donor physician for NMDP donors. Donors must meet standard eligibility criteria for donation of blood or marrow. They will be screened with the standard blood bank donor questionnaire, medical history and testing for infectious disease markers by a physician who is experienced in screening transplant donors. Only donors who have cleared this process and deemed to be eligible will provide blood for CTL generation.

#### 2.3.5 Donor Evaluation

2.3.5.1. Complete history and physical examination

2.3.5.2 CBC, platelets, differential

2.3.5.3. Electrolytes, BUN, creatinine, glucose, total protein, albumin, total bilirubin, alkaline phosphatase, ALT, AST, LDH, serum protein electrophoresis (if indicated)

2.3.5.4. HIV-1 antibody, HIV-2 antibody, HIV NAT, HTLV-1/2 antibodies, HBs antigen, HBc antibody, HCV NAT, CMV antibody, RPR, West Nile virus NAT, and Chagas testing

2.3.5.5 ABO and Rh typing

2.3.5.6. Hemoglobin electrophoresis or Sickle Prep test (if indicated)

2.3.5.7. Complete urinalysis

2.3.5.8 When the evaluation is complete, the Transplant Physician will note in the recipient's and donor's medical records that the tests have been evaluated, and the donor is acceptable.

## 2.4 Treatment Plan

Patients may be screened for study entry when they have persistent disease despite standard therapy for 5 days. At that stage a search will be done of the available lines. Lines were generated from HSCT donors who consented to the use of CTLs not required for their recipient for research or from normal donors. All donors were screened and deemed to be eligible as transplant donors. We will also manufacture additional lines with the goal of covering common HLA types and will consult with the NMDP to determine what HLA types would be desirable. Additional donors will be screened by a transplant donor center physician and must be deemed eligible before a line can be manufactured. (see 2.3.4 and 2.4.5)

### 2.4.1 CTL Lines

We will use trivirus specific CTL lines generated as described previously.<sup>24</sup> Generation of trivirus-specific CTL lines requires the generation of several different components from PBMC. The CTL line will be derived from donor peripheral blood T cells, by multiple stimulations with antigen-presenting cells (APCs) presenting CMV, EBV and adenovirus antigens and expansion with interleukin-2 (IL-2). The APCs used to stimulate and expand the CMV-specific T cells will be derived from patient mononuclear cells and B lymphocytes.

To initiate the trivirus-specific CTL line, PBMC will be transduced with an adenovirus vector (Ad5f35-pp65) expressing the immunodominant antigen of CMV, pp65. The monocyte fraction

of PBMC expressed and presented CMV-pp65 peptide epitopes to the CMV-specific T cell fraction of the PBMC, while the virion proteins from the adenovirus vector were processed and presented to the adenovirus-specific T cell fraction.

To expand trivirus -specific T cells we used EBV-transformed B lymphoblastoid cell lines (EBV-LCLs) transduced with Ad5f35-pp65. This transduction allows the EBV-LCLs to present CMV-pp65 and adenovirus virion peptides to the T cells as well as endogenously expressed EBV antigens.

EBV-LCLs are derived from PBMC-B lymphocytes by infection with a clinical grade, laboratory strain of Epstein-Barr virus (EBV). About  $5 \times 10^6$  PBMC, or 5 to 10 mLs of blood is required to generate the EBV-LCL

At the end of the CTL culture period, the frequency of T cells specific for each virus were determined using tetramer reagents if available. To test the functional antigen specificity of the CTL we will use overlapping peptide libraries for pp65 and adenovirus hexon and autologous and allogeneic LCLs in Elispot assays and we will perform cytotoxicity assays using unmodified PHA blasts and LCLs untransduced or transduced with Ad5f35-null and Ad5f35-pp65.

The CTL lines will also be checked for identity, phenotype and sterility, and cryopreserved prior to administration according to SOP. Release criteria for administering the CTL to patients include viability >70%, negative culture for bacteria and fungi for at least 7 days, endotoxin testing  $\leq 5$  EU/ml, negative result for Mycoplasma, <2% CD19 positive B cells, <2% CD14 positive monocytes (or <2% CD83 positive cells if Dendritic cells were used as stimulators) and HLA identity.

#### *2.4.2 No Matched CTL Line Available*

If no matched line is available the patient will be registered so that the feasibility of the approach can be assessed and the eventual outcome will also be collected.

#### *2.4.3 Criteria for Selection of CTL Line*

In general the line matching at the highest number of HLA loci will be selected. Matching at the allele level will be preferred but antigen level will be accepted for HLA-A and HLA-B. However consideration will also be given to the type of infection and activity of the line against that virus. For example for a patient with adenovirus infection a line that matches at 2 loci but that has recognition of adenovirus mediated through those antigens would be preferable to a line matched at 3 loci but with no demonstrated activity against adenovirus. The protocol chair will discuss each case with the principal investigators at each center to determine the optimal CTL line for each patient. If more than one line matches and there are insufficient cells to cover additional infusions a second CTL line will be reserved in the event that additional infusions are warranted. Patients with a partial response are eligible to receive an additional dose.

#### *2.4.4 Shipping of CTL Line*

The line will be shipped from the Production Assistance for Cellular Therapies (PACT) center at Baylor College of Medicine (BCM) to the clinical center using validated shipping methodology. The line will be accompanied by a certificate of analysis and an SOP for administration.

## 2.4.5 Administration and Monitoring

2.4.5.1 Multivirus specific T cells will be thawed and given by intravenous injection.

2.4.5.2 Premedications:

Patients will be premedicated with Benadryl 1mg/kg (max 50 mg) IV and Tylenol 10 mg/kg (max 650 mg) PO.

2.4.5.3 Patients will be monitored according to institutional standards for administration of blood products and at a minimum will be monitored according to below:

- Patients should remain in the clinic for at least one hour
- Patients should remain on continuous pulse oximetry for at least 30 minutes
- Vital signs should be monitored at the end of infusion then at 30 and 60 minutes

2.4.5.4. Supportive Care: Patients will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other intervention as appropriate.

2.4.5.5 If a patient has a partial response (as defined in 3.2.1) they are eligible to receive up to 4 additional doses at biweekly intervals. These doses would come from the original infused line if sufficient vials were available but may come from another line if there are insufficient cells in the original line.

## 2.4.6 Risks and Toxicities

2.4.6.1 Graft Versus Host Disease (GVHD)

The risk that adoptively transferred partially HLA matched CTL will cause GVHD is very low according to published data from previous studies.<sup>24;27</sup>

2.4.6.2 GVHD Scoring

Weekly GVHD organ stage scores, overall clinical grade, biopsy information for GVHD and relevant differential diagnosis will be recorded. The weekly score will encompass all information since the last assessment. Organ involvement, biopsy information, staging, differential diagnosis, and GVHD therapy will be documented in the medical record using the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) GVHD scoring stamp or equivalent.

Symptoms of chronic GVHD, if present, will be reported according to the SCCT MOP and reported on the GVHD symptom record.

An example acute GVHD weekly data record (stamp) is shown below.

Clinical Acute GVHD Assessment													
Date _____			Patient ID _____				Karnofsky/Lansky _____						
<b>CODES</b>						<b>DIFFERENTIAL DIAGNOSIS</b>							
	0	1	2	3	4	5	GVHD	Drug Rxn	Cond Reg	TPN	Infect	VOD	Other
Skin	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lower GI	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Upper GI	<input type="checkbox"/>	<input type="checkbox"/>					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Liver	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Treatment:</b> <input type="checkbox"/> CSA <input type="checkbox"/> Tacrolimus <input type="checkbox"/> Pred <input type="checkbox"/> Methylpred <input type="checkbox"/> Ontak <input type="checkbox"/> Pentostatin <input type="checkbox"/> MMF <input type="checkbox"/> Etanercept <input type="checkbox"/> Other _____													
<b>Code Definitions:</b>													
<u>Skin:</u>			<u>Lower GI (Diarrhea):</u>			<u>Upper GI:</u>			<u>Liver (Bilirubin):</u>				
0 No rash			0 None			0 No protracted nausea and vomiting			0 < 2.0 mg/dl				
1 Maculopapular rash, < 25% of body surface			1 ≤ 500 mL/day or < 280 mL/m <sup>2</sup>			1 Persistent nausea, vomiting or anorexia			1 2.1-3.0 mg/dl				
2 Maculopapular rash, 25-50% of body surface			2 501-1000 mL/day or 280- 555 mL/m <sup>2</sup>						2 3.1-6.0 mg/dl				
3 Generalized erythroderma			3 1001-1500 mL/day or 556- 833 mL/m <sup>2</sup>						3 6.1-15.0 mg/dl				
4 Generalized erythroderma with bullous formation and desquamation			4 > 1500 mL/day or > 833 mL/m <sup>2</sup>						4 > 15.1 mg/dl				
			5 Severe abdominal pain with or without ileus, or stool with frank blood or melena										
Signature _____													

Chronic GVHD

Patients developing sign/symptoms of chronic GVHD (CGVHD) will have symptoms recorded on the CGVHD scoring form at the scheduled follow-up visits.

**Definite and Possible Manifestations of Chronic GVHD**

<b>Organ System</b>	<b>Definite manifestations of chronic GVHD</b>	<b>Possible manifestations of chronic GVHD</b>
Skin	Scleroderma (superficial or fasciitis), lichen planus, vitiligo, scarring alopecia, hyperkeratosis pilaris, contractures from skin immobility, nail bed dysplasia	Eczematoid rash, dry skin, maculopapular rash, hyperpigmentation, hair loss
Mucous membranes	Lichen planus, non-infectious ulcers, corneal erosions/non-infectious conjunctivitis	Xerostomia, keratoconjunctivitis sicca
GI tract	Esophageal strictures, steatorrhea	Anorexia, malabsorption, weight loss, diarrhea, abdominal pain
Liver	None	Elevation of alkaline phosphatase, transaminitis, cholangitis, hyperbilirubinemia
GU	Vaginal stricture, lichen planus	Non-infectious vaginitis, vaginal atrophy
Musculoskeletal/ Serosa	Non-septic arthritis, myositis, myasthenia, polyserositis, contractures from joint immobilization	Arthralgia
Hematologic	None	Thrombocytopenia, eosinophilia, autoimmune cytopenias
Lung	Bronchiolitis obliterans	Bronchiolitis obliterans with organizing pneumonia, interstitial pneumonitis

2.4.6.2 *Other toxicities:* Should unanticipated toxicities arise (e.g. severe local reactions or hepatorenal damage) they, too, will be graded by the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0.

2.4.6.3 *Management of Toxicity.* CTLs are susceptible to killing by steroids give at a dose of 1-2mg/kg. This is standard therapy for GVHD and could also be given if a recipient develops other complications considered possibly related to CTL administration. Other supportive care would be per standard medical practice.

## CHAPTER 3

### 3 STUDY ENDPOINTS

#### 3.1 Primary Endpoint

The primary objectives are feasibility and safety. The primary endpoint is to assess the safety of administration of CTLs 45 days post-infusion. The safety endpoint will be defined as acute GvHD grades III-IV within 45 days of the last dose of CTLs or grades 3-5 infusion-related adverse events within 30 days of the last CTL dose or grades 4-5 nonhematological adverse events within 30 days of the last CTL dose and that are not due to the pre-existing infection or the original malignancy or pre-existing co-morbidities as defined by the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0. Toxicities to consider include GI toxicity, renal toxicity, hemorrhagic toxicity, cardiovascular toxicity (hypotension, cardiac arrhythmia and left ventricular systolic dysfunction, neurologic toxicity (somnolence and seizure), coagulation toxicity, vascular toxicity and pulmonary toxicity.

#### 3.1.1 Staging and Grading of Acute GVHD

##### Staging\*

	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Skin	No rash	Rash < 25% BSA	25-50%	> 50% Generalized erythroderma	Plus bullae and desquamation
Gut	< 500 mL diarrhea/day	501-1000 mL/day	1001–1500 mL/day	> 1500 mL/day	Severe abdominal pain & ileus
UGI		Severe nausea/vomiting			
Liver	Bilirubin ≤ 2 mg/dl	2.1-3 mg/dl	3.1-6mg/dl	6.1-15mg/dl	> 15 mg/dl

Acute GVHD grading should be performed by the consensus conference criteria (Przepiorka, et al., 1994).<sup>32</sup>

##### Grading Index of Acute GVHD\*

	Skin	Liver	Gut	Upper GI
0	None and	None and	None and	None
I	Stage 1-2 and	None and	None	None
II	Stage 3 and/or	Stage 1 and/or	Stage 1 and/or	Stage 1
III	None-Stage 3 with	Stage 2-3 or	Stage 2-4	N/A
IV	Stage 4 or	Stage 4	N/A	N/A

## 3.2 Secondary Endpoints

### 3.2.1 Antiviral Activity

Viral load will be monitored for CMV, EBV and adenovirus. For the infection under treatment response in viral load will be defined as follows:

**Complete response:** Return to normal range as defined by specific assay used.

**Partial response:** Decrease in viral load of at least 50% from baseline.

**Stable disease:** Changes insufficient to qualify as partial response or progression

**Progression:** Increase in viral load of at least 50% from baseline.

### 3.2.2 Reconstitution of Antiviral Immunity

Patients will be monitored using ELISPOT assays or tetramer assays with appropriate viral specific peptide mixtures.

### 3.2.3 Persistence of Infused CTLs

Persistence of infused T cells will be monitored based on quantitative PCR for non-shared HLA antigens.

### 3.2.4 Effects on Clinical Signs of Viral Infection.

If a patient has organ involvement clinical response will be monitored.

### 3.2.5 Survival

Overall survival at 6 and 12 months post CTL infusion will be computed.

### 3.2.6 Chronic GVHD

Chronic GVHD will be assessed at 6 and 12 months post CTL infusion.

### 3.2.7 Systemic Infections

All microbiologically documented infections occurring within 6 months of CTL infusion will be reported by etiologic agent, site of disease, date of onset, and severity.

### 3.2.8 Secondary graft failure

Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in the ANC to  $< 500/\text{mm}^3$  for three consecutive measurements on different days, unresponsive to growth factor therapy that persists for at least 14 days. Secondary graft failure will be assessed at 30 days post CTL infusion.



## CHAPTER 4

### 4 PATIENT ENROLLMENT AND EVALUATION

#### 4.1 Enrollment

Patients will be registered as follows:

1. An authorized user at the clinical center completes the initial screening when the patient has an adenovirus, CMV or EBV infection ***persistent despite standard treatment as defined*** and enrolls the patient in AdvantageEDC<sup>SM</sup> system on the screening phase of the study (Segment A) after obtaining the screening consent.
2. A search is done to determine if there is a suitably matched line using the AdvantageEDC<sup>SM</sup> system.
3. If a CTL line is not available, the following data will be collected: demographic data, HLA type, infection type and follow up data using the Transplant Essential Data form sent to the Stem Cell Transplant Outcomes Database at 100 days and 1 year.
4. If a CTL is available, the protocol chair or designee discusses the available line with the principal investigator at the center. An authorized user at the transplant center will request the CTL. If the CTL is confirmed by the Data Coordinating Center (DCC), the transplant center completes the Demographics Form and the Segment B enrollment Form in AdvantageEDC<sup>SM</sup>. The eligibility screening (Segment B) includes a question confirming that the patient signed the treatment informed consent form.
5. If the patient is eligible, a study number and CTL assignment number are generated on the "Enrollment Successful" page. This page should be printed immediately as it is the only opportunity to do such. An email will be sent to the Cell Therapy lab to initiate the processing and shipping to the transplant center.
6. A visit schedule based on treatment start date is displayed for printing and is referred to as "Segment B Follow-up."

If a connection is interrupted during an enrollment session, the process is completely canceled and logged. A backup manual enrollment system will also be available to provide for short-term system failure or unavailability.

#### 4.2 Study Monitoring

##### 4.2.1 Follow-up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 4.2.1.

**Follow-up Assessments:** The timing of follow-up visits is based on the date of CTL infusion. If a patient has multiple CTL doses the schedule resets again at the beginning so follow up relates to the last CTL dose.

**Reporting Patient Deaths:** The Recipient Death Information must be entered into the web-based data entry system within 24 hours of knowledge of a patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated.

**Table 4.2.1  
FOLLOW-UP SCHEDULE**

<b>Assessment Time</b>	<b>Target Day<sup>1</sup> (Days Post-Enrollment)</b>
1 week	7 days
2 weeks	14 days
3 weeks	21 days
4 weeks	28 days
6 weeks	42 days
90 days	90 days
6 months	180 days
12 months	365 days

<sup>1</sup>Target day range =  $\pm 2$  days up to Week 8, and  $\pm 14$  days for Days 90, 120, and  $\pm 28$  days for 6 and 12 months, post-randomization.

#### 4.2.2 Assessments

All assessments are considered standard-of-care unless identified below by “\*.”

##### Pre-Infusion

1. History and physical exam including height and weight
2. Viral loads for EBV, adenovirus, CMV
3. Biopsy disease site, if appropriate
4. Imaging studies, if appropriate
5. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests
6. CBC with differential, platelet count
7. Liver function tests (bilirubin, alkaline phosphatase, AST, ALT) plus creatinine
8. Tacrolimus/cyclosporine level
9. Samples for laboratory studies

##### Post-Infusion

1. Viral loads for CMV, EBV, adenovirus weekly until Day 42, and 3, 6 and 12 months.

2. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests weekly until Day 42, and 3, 6 and 12 months
3. Chronic GVHD evaluation (if present) 3, 6 and 9 months
4. CBC with differential and platelet count weekly until Day 42
5. Toxicity evaluation weekly until Day 42
6. Steroid dose weekly until Day 42, and 3, 6 and 9 months
7. Samples for laboratory studies on Days 0, 14, 28 and 90
8. Infections through Day 42 and at 3, 6 and 12 months

**Table 4.2.2 REQUIRED ASSESSMENTS**

	Days Post-CTL Infusion										
	Screening	Baseline (Day 0)	7	14	21	28	42	90	180	270	365
Initiate search for CTL line	X										
Suggested biopsy of involved tissue if appropriate		X									
History and physical exam		X									
CMV, EBV, adenovirus load		X	X	X	X	X	X	X	X	X	X
Acute GVHD evaluation		X	X	X	X	X	X	X	X	X	X
Chronic GVHD evaluation								X	X	X	X
CBC with differential, platelet count		X	X	X	X	X	X				
Basic chemistry (creatinine)		X	X	X	X	X	X <sup>2</sup>	X <sup>2</sup>	X <sup>2</sup>	X <sup>2</sup>	X <sup>2</sup>
Liver function tests (alkaline phosphatase, bilirubin, AST, ALT)		X	X	X	X	X	X	X	X	X	X
Toxicity evaluation			X	X	X	X	X				
Steroid dose			X	X	X	X	X	X	X	X	X
Blood and serum for ancillary laboratory studies		X <sup>1</sup>		X <sup>1</sup>		X <sup>1</sup>		X <sup>1</sup>			

<sup>1</sup> Research procedures beyond that required for usual care. (30-40 mls or 6-8 teaspoons will be collected at each timepoint)

<sup>2</sup> Recommended

Ancillary laboratory studies will include

- 1) Assessment of virus-specific immunity based on CTL levels as measured by ELISPOT assays or tetramer assays.
- 2) Persistence of infused T cells based on PCR for non-shared antigen
- 3) Serum will be stored at baseline for possible future PRA testing if the allogeneic CTLs do not persist

#### 4.2.3 Adverse Event (AE) /Serious Adverse Event (SAE) Reporting

##### 4.2.3.1. Adverse Event Definitions

Adverse Event - Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of definite, probable, possible, unlikely, or unrelated).

Life-Threatening Adverse Event - Any adverse event that places the participant, in view of the investigator, at immediate risk of death from the reaction.

Serious Adverse Event (SAE) - Any adverse event that results in any of the following outcomes:

- death,
- a life threatening adverse event,
- in-patient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity,
- a congenital anomaly/birth defect, or
- any other medical event, in appropriate medical judgment, may require medical or surgical intervention to prevent one of the outcomes listed above.

Unexpected Adverse Event - For SCCT studies involving hematopoietic stem cell transplantation: any adverse event, the specificity or severity of which is NOT listed in the study protocol, product inserts or informed consent document.

Attribution - The determination of whether an adverse event is related to a drug/device/treatment. Attribution categories:

Definite-The adverse event is **clearly related** to the study drug/device/procedure/ treatment(s).

Probable-The adverse event is **likely related** to the study drug/device/procedure treatment.

*For SCCT studies involving hematopoietic stem cell transplantation: the adverse event is not likely to be caused by the subject's underlying medical condition or other concomitant therapy, and the nature of the adverse event or the temporal relationship between the onset*

*of the adverse event and study drug/device/treatment administration lead the investigator to believe that there is a reasonable chance of causal relationship.*

Possible -The adverse event **may be related** to the study drug/device/procedure/ treatment(s).

*For SCCT studies involving hematopoietic stem cell transplantation: the adverse event could be attributed to the subject's underlying medical condition or other concomitant therapy, but the nature of the adverse event or the temporal relationship between the onset of the adverse event and study drug/device/treatment administration lead the investigator to believe that there could be a causal relationship.*

Unlikely -The adverse event is **doubtfully related** to the study drug/device procedure/treatment(s).

Unrelated -The adverse event is **clearly NOT related** to the study drug/device/procedure/ treatment(s).

*For SCCT studies involving hematopoietic stem cell transplantation: the adverse event is most plausibly explained by the subject's underlying medical condition or other concomitant therapy, or the adverse event has no plausible biological relationship to study drug/device /treatment.*

Common Terminology Criteria Adverse Events (CTCAE) – a descriptive terminology developed by the National Cancer Institute (NCI) for use in reporting adverse events. The CTCAE includes a grading (severity) scale for each adverse event term. A copy of the current CTCAE guidelines (Version 3.0) is located at <http://ctep.cancer.gov/reporting/>.

Grade – Severity of the adverse event. Grades were developed using the following guidelines:

- Grade 0 – No adverse event or within normal limits
- 1 – Mild adverse event
- 2 – Moderate adverse event
- 3 – Severe adverse event
- 4 – Life-threatening or disabling adverse event
- 5 – Fatal adverse event

Abnormal laboratory values not included in the CTCAE guidelines will be defined per protocol.

#### Time for AE Collection

All adverse events will be collected for 30 days after the last CTL infusion

Graft versus host disease evaluation will be collected for 45 days after the last CTL infusion

.

#### 4.2.3.2. Adverse Event Reporting

Adverse events should be reported using CTCAE terminology and severity scales for all SCCT studies involving hematopoietic stem cell transplantation. Information reported for the adverse event must include: Name of adverse event, date of first onset, peak severity, relationship to study drug/device/treatment, resolution date, actions taken with respect to administration of

study drug/device/treatment, and other treatment for the adverse event. Table 4.2.3.4 describes reporting time frames. Adverse events will be reported as long as specified in the protocol.

Any adverse event that is sent to the local IRB will be sent at the same time to the DCC. The DCC will ensure that the report contains sufficient information, will code it using MEDRA, and will review and distribute as described below.

#### 4.2.3.3. SCCT Monitoring of Adverse Events

Table 4.2.3.4 summarizes the reporting requirements for expected and unexpected adverse events. The section below describes in more detail the reporting requirements.

### **Unexpected Grades 3-5 Adverse Events**

- All unexpected Grades 3-5 adverse events will be reviewed by the Medical Monitor at the DCC, within 2 business days of receiving the adverse event form (or MedWatch form) from the clinical center.
- If the Medical Monitor requires additional information to make his/her assessment, the clinical centers will have 2 business days to respond to the request for additional information.
- The DCC is responsible for notifying the NHLBI Project Officer, or designated NHLBI medical monitor, immediately of all unexpected Grades 3-5 adverse events, regardless of attribution, and of any concerns regarding the frequency or type of adverse event(s) on a study or study treatment arm.
- The attribution, as assessed by the clinical center and the DCC medical monitor, will be provided to the NHLBI Project Officer or designated NHLBI medical monitor, within 2 business days but no later than 7 days of receiving the report.
- All unexpected Grades 3- 5 adverse events will also be sent to the DSMB.

The NHLBI Project Officer (or designee) is responsible for reviewing the adverse event materials to determine if the materials are complete. If there are any concerns regarding the type or frequency of the event, the NHLBI Project Officer will request that the DSMB Executive Secretary notify the DSMB. The DSMB will review the adverse event materials, determine if the information is complete, determine if additional DSMB review is required and make recommendations to the NHLBI concerning continuation of the study.

The DCC will prepare quarterly summary reports of all unexpected Grades 3-5 adverse events and annual summary reports of all unexpected Grades 1-2 for the NHLBI Project Officer (or designee) and DSMB. Quarterly reports will be made available on a secure website and the NHLBI Project Officer (or designee) and DSMB will be notified by e-mail when the materials are posted. The summary reports will include a minimum of:

- description of the adverse event,
- whether it is expected or unexpected,
- the degree of attribution,
- a clinical summary and conclusion.



### **Expected Adverse Events**

- All expected Grade 5 adverse events will be reviewed by the Medical Monitor at the DCC, within 1 business day of receiving the adverse event form (or MedWatch form) from the clinical center.
- If the Medical Monitor requires additional information to make his/her assessment, the clinical centers will have 2 business days to respond to the request for additional information.
- The DCC is responsible for notifying the NHLBI Project Officer, or designated NHLBI medical monitor, immediately of all expected Grades 5 adverse events, regardless of attribution, and of any concerns regarding the frequency or type of adverse event(s) on a study or study treatment arm.
- The attribution, as assessed by the clinical center and the DCC medical monitor, will be provided to the NHLBI Project Officer or designated NHLBI medical monitor, within 2 business days, but no later than 7 days of receiving the report.
- All expected Grade 5 adverse events will also be sent to the DSMB.

The DCC will prepare quarterly summary reports of all expected Grades 3-5 adverse events and annual summary reports of all expected Grades 1-2 for the NHLBI Project Officer (or designee) and DSMB. The reports will be made available on a secure website and the NHLBI Project Officer (or designee) and DSMB will be notified by e-mail when the materials are posted. The summary reports will include a minimum of:

- description of the adverse event,
- whether it is expected or unexpected,
- the degree of attribution,
- a clinical summary and conclusion.

Any concern regarding the type or frequency of a Grade 3-5 expected adverse event will be reported to the NHLBI Project Officer who will determine if referral to the DSMB is warranted. If required, data materials will be provided by the DCC. The DSMB Executive Secretary will arrange for review by the DSMB Chair. The Chair will determine if additional DSMB review is required and make recommendations to the NHLBI concerning continuation of the study.

#### 4.2.3.4. FDA IND/IDE Reporting

The sponsor of the IND/IDE is responsible for reporting to the FDA on at least an annual basis and for reporting expedited safety reports (21 CFR 312.32). Annual reports are due within 60 days of the IND/IDE submission anniversary. If a study is under an FDA IND or IDE, all unexpected adverse events are reported to the FDA by telephone or fax as defined in Table 4.2.3.4 below. Reporting will be expedited for SAEs as defined in section 4.2.3.1 that are unexpected, any increase in the rate or severity of expected toxicities, and any grades 3-5 infusion-related adverse events.

**Table 4.2.3.4 Reporting Requirements**

Relationship	Grade	Attribution	Clinical Center Reporting Requirements to the DCC	DCC Reporting to NHLBI	FDA Reporting
Unexpected	1-2	Unrelated/ Unlikely	Summarized annually	Summarized annually	Summarized annually
		Possible/ Probable/ Definite			
	3-5	Unrelated/ Unlikely	Report to the DCC within 1 business day.	Grades 3-5 within 2 calendar days but no later than 7 days	Grades 4-5 within 7 calendar days by telephone or fax.  Grades 3-5 written report within 15 calendar days
		Possible/ Probable/ Definite			
Expected	1-2	Unrelated/ Unlikely	Report to the DCC quarterly. Reportable events in this category will be defined on a study specific basis and captured on case report forms.	Summarized annually	Summarized annually
		Possible/ Probable/ Definite			
	3-4	Unrelated/ Unlikely		Summarized quarterly	
		Possible/ Probable/ Definite			
	5	Any		Report to the DCC within 1 business day.	
<p><b>PLEASE NOTE:</b> Any adverse event that is sent to the local IRB will be sent at the same time to the DCC. Grade 5 means a fatal event has occurred.</p>					

#### 4.3 Off Study Criteria

Patients will be off study if

- 4.3.1. They receive any other hemopoietic cell product
- 4.3.2 They receive therapy for relapse of their primary malignancy
- 4.3.3 Refusal of further study follow up by patient or legal guardian

In a patient comes off study to receive other hemopoietic cell products or therapy of malignancy we will continue to collect data on long term follow up for safety of gene transfer.

## CHAPTER 5

### 5 STATISTICAL CONSIDERATIONS

#### 5.1 Study Design Synopsis

This study is a multi-center, Phase I/II trial designed to evaluate the feasibility, safety and efficacy of most closely HLA matched multivirus specific CTL lines (CHM-CTLs) in HSCT patients with either of three viruses; EBV, CMV and adenovirus persistent despite standard therapy. Eighteen patients will be initially enrolled in the phase I study to assess safety in cohorts of size 6 for CMV, EBV and adenovirus at a standard dose of  $2 \times 10^7$  CTLs/m<sup>2</sup>. Safety and toxicity will be evaluated at the completion of each cohort before proceeding onto the phase II portion. For example, once 6 patients are followed and evaluated for safety and toxicity on the CMV cohort, a decision will be made to continue to the phase II portion independent of the other two cohorts. Phase II will consist of 27 patients in cohorts of size 9 for CMV, EBV and adenovirus at a standard dose of  $2 \times 10^7$  CTLs/m<sup>2</sup>. The total sample size of the study will be 45 patients on the treatment arm. A larger number of potential subjects will be screened to assess the feasibility of the approach.

#### 5.2 Accrual

It is estimated that 24 months of accrual, or approximately 2 patients per month, will be necessary to enroll the targeted sample size.

#### 5.3 Primary Endpoint

The primary endpoint for the trial is to determine the safety of banked allogeneic viral specific CTLs within 45 days of last dose of CTLs. Events to be considered include grade III-IV acute graft-versus-host disease (aGvHD) within 45 days of the last dose of CTLs or grades 3-5 infusion-related adverse events or grades 4-5 nonhematological adverse events within 30 days of the last CTL dose and that are not due to the pre-existing infection or the original malignancy as defined by the National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

#### 5.4 Sample Size and Power Calculations

The sample size is 45 patients for this trial. Ninety-five percent confidence intervals were calculated for varying probabilities based on the sample size. Table 5.4.1 provides confidence intervals for a variety of true underlying proportions. Of particular interest is the anticipated 30 day acute GVHD or grade 3 or higher toxicity, which is 40%. For this setting, the confidence interval length is 28.6%. The probabilities above and below 40% represent other plausible safety scenarios. Sample sizes of 15 and 30 are provided in the event a cohort is closed early.

The precision of the estimates alternatively could be viewed as a lower bound on the rate of the safety endpoint. The probability to rule out safety events of a certain size can be interpreted as "power." Table 5.4.2 provides the probability (or power) that the lower bound of a 95% two-sided confidence interval for the safety event probability will be greater than thresholds of 30%, 50%, 60%, 65% and 70%. When the true percentage is 40%, there is 91% power to rule out a percentage of 65% or larger.

**Table 5.4.1**  
**Confidence interval lengths and possible confidence intervals for various sample sizes and underlying safety event proportions**

N	Event Proportion %	Length of 95% Confidence Interval	Possible Confidence Interval
45	30	26.8	16.6, 43.4
	35	27.9	21.1, 48.9
	40	28.6	25.7, 54.3
	45	29.1	30.5, 59.5
	50	29.2	35.4, 64.6
30	30	32.8	13.6, 46.4
	35	34.1	17.9, 52.1
	40	35.1	22.5, 57.5
	45	35.6	27.2, 62.8
	50	35.8	32.1, 67.9
15	30	46.4	6.8, 53.2
	35	48.3	10.9, 59.1
	40	49.6	15.2, 64.8
	45	50.4	19.8, 70.2
	50	50.6	24.7, 75.3

**Table 5.4.2**  
**Probability of ruling out a threshold of size T or larger for various sample sizes and true underlying safety event proportion**

N	True Event Proportion %	Probability of Ruling Out Event Percentages of Size T or Larger					
		T=30%	T=40%	T=50%	T=60%	T=65%	T=70%
45	30		0.26	0.75	0.97	1.00	1.00
	35	0.07	0.09	0.48	0.88	0.98	1.00
	40	0.22		0.23	0.68	0.91	0.98
	45	0.47	0.06	0.08	0.41	0.75	0.90
	50	0.72	0.19		0.19	0.50	0.72
30	30		0.16	0.59	0.92	0.96	0.99
	35	0.07	0.06	0.36	0.78	0.87	0.97
	40	0.18		0.18	0.58	0.72	0.90
	45	0.36	0.08	0.07	0.36	0.50	0.77

	50	0.57	0.18		0.18	0.29	0.57
15	30		0.04	0.30	0.52	0.72	0.87
	35	0.04	0.02	0.17	0.35	0.56	0.76
	40	0.10		0.09	0.22	0.40	0.61
	45	0.18	0.03	0.05	0.12	0.26	0.45
	50	0.30	0.06		0.06	0.15	0.30

## 5.5 Stopping Guidelines

Statistical monitoring of aGVHD grades III-IV or grade 3-5 toxicity and secondary graft failure is employed in this study in two phases. The guideline is designed to assist an independent NHLBI-appointed Data and Safety Monitoring Board (DSMB) in overseeing the study. The DSMB may also request additional interim analyses and develop other criteria for determining when to intervene in the enrollment or treatment of patients in the study. Monitoring of key safety endpoints will be conducted and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised.

Safety guidelines will be implemented separately for each cohort. The risk of toxicity or aGVHD is considered to be moderate in this population. Three reports of grade III aGVHD or grade 3 toxicity in the cohort of 6 will stop enrollment to that cohort and not continue on to phase II.

The proportion of events by 30 days post CTL infusion will be monitored using a stopping guideline that compares the rate of events to an external standard for each cohort. A Bayesian motivated safety stopping guideline will be used for this trial. The expected underlying 30 day post-CTL infusion event probability is assumed to be 25% and that a probability of greater than 65% is unacceptable. A Beta distribution can be used as the prior distribution of  $\Theta$ ;  $\Theta$  is the proportion of patients who experience an event. The Beta distribution will have prior mean of 0.25 and a prior probability  $<0.05$  of exceeding 0.45. The guideline is derived such that if there is strong evidence (posterior probability  $\geq 0.95$ ) that the probability of the event is greater than 25%, the trial will be stopped. The resulting boundaries are tabulated in Table 5.5.1.

**TABLE 5.5.1**

**Bayesian Stopping Guideline for Event Rate of 25% \***

# Events	# Patients in Cohort
4	6
5	7-8
6	9-11
7	12-14
8	15

\* The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment.

A simulation study was conducted to evaluate the operating characteristics of this stopping rule. Data were generated from the binomial distribution with varying probabilities of failure ( $\Theta$ ) and assuming a sample size of 15 patients. Table 5.5.2 shows the probability of stopping the trial early and the average sample size of stopped trial (N), conditional on stopping early, at which the boundary is crossed for each value of  $\Theta$ .

**Table 5.5.2**

**Operating Characteristics for Bayesian Motivated Stopping Guideline**

Mean of Prior Distribution	$\theta$	Probability of stopping	Average Sample Size of Stopped Trial (N)
0.25	0.25	0.07	9
	0.35	0.25	9
	0.45	0.53	9
	0.55	0.79	8
	0.65	0.94	8

Although the motivation for the boundary is Bayesian, the operating characteristics can be evaluated from a frequentist perspective of Type I error and power. The stopping rule for a 25% event rate has a 7% chance (“Type I error”) of suggesting early termination when the true rate is 0.25, and an 94% chance (“power”) when the true rate is 0.65.

The proportion of secondary graft failures by 30 days post CTL infusion will be monitored using a stopping guideline that compares the rate of events to an external standard for each cohort. A Bayesian motivated safety stopping guideline will be used for this trial. The expected underlying 30 day post-CTL infusion secondary graft failure probability is assumed to be 5% and that a probability of greater than 35% is unacceptable. A Beta distribution can be used as the prior distribution of  $\Theta$ ;  $\Theta$  is the proportion of patients who experience an event. The Beta distribution will have prior mean of 0.05 and a prior probability <0.05 of exceeding 0.35. The guideline is derived such that if there is strong evidence (posterior probability  $\geq 0.95$ ) that the probability of the event is greater than 5%, the trial will be stopped. The resulting boundaries are tabulated in Table 5.5.1.

**TABLE 5.5.3**

**Bayesian Stopping Guideline for Event Rate of 5% \***

# Events	# Patients in Cohort
3	6-9
4	10-15

\* The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment.

A simulation study was conducted to evaluate the operating characteristics of this stopping rule. Data were generated from the binomial distribution with varying probabilities of failure ( $\Theta$ ) and

assuming a sample size of 15 patients. Table 5.5.2 shows the probability of stopping the trial early and the average sample size of stopped trial (N), conditional on stopping early, at which the boundary is crossed for each value of  $\Theta$ .

**Table 5.5.4**

**Operating Characteristics for Bayesian Motivated Stopping Guideline**

Mean of Prior Distribution	$\theta$	Probability of stopping	Average Sample Size of Stopped Trial (N)
0.05	0.05	0.01	9
	0.15	0.22	9
	0.25	0.58	9
	0.35	0.85	8
	0.45	0.96	7

Although the motivation for the boundary is Bayesian, the operating characteristics can be evaluated from a frequentist perspective of Type I error and power. The stopping rule for a 5% event rate has a 1% chance ("Type I error") of suggesting early termination when the true rate is 0.05, and an 85% chance ("power") when the true rate is 0.35.



## 5.6 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Covariates considered will include age, race, sex, type of donor, graft source, HLA match of transplant, HLA match of CTL, type of infection, site of infection, aGVHD grade at enrollment, and time from transplantation to infusion of CTLs.

## 5.7 Endpoints and Analysis Plan

All endpoints are assessed from the last dose of CTL. The starting time point for each analysis will initiate at the start of the infusion of the last dose. The primary endpoint is the proportion of patients with aGVHD grades III-IV within 45 days of the last dose of CTLs or grades 3-5 infusion-related adverse events within 30 days of the last dose of CTLs or grades 4-5 nonhematological adverse events within 30 days of the last dose of CTLs and that are not due to the pre-existing infection or the original malignancy or pre-existing co-morbidities. Descriptive analyses of the primary endpoint will be performed. Point estimates and confidence intervals of safety event proportion will be computed. Event rates will be compared across infection cohorts. A test of homogeneity will be conducted across cohorts with respect to the primary endpoint.

Secondary endpoints include:

- Effects of CHM-CTL on viral loads.
- Reconstitution of antiviral immunity at 2 weeks, 1 and 3 months.
- Persistence of infused CHM-CTLs and effects on clinical signs of viral infection.
- Cumulative incidence of viral reactivations within 6 months.
- Cumulative incidence of secondary graft failure at 30 days.
- Cumulative incidence of systemic infections within 6 months.
- Cumulative incidence of chronic GVHD at 6 and 12 months.
- Clinical response to CTL infusions at 5 weeks and 3 months after the start of CTL infusions. Response will be graded as complete, partial, stable disease, progression, and did not respond as defined in Chapter 3.
- Effects of HLA matching.
- Overall survival at 6 and 12 months post CTL infusion. Overall survival will be estimated based on the Kaplan-Meier product limit estimator. Death will be considered the event and censored on the last date of follow-up. Survival probabilities and confidence intervals will be calculated.

Estimates and 95% confidence intervals will be constructed for all endpoints. These may include point estimates, Kaplan-Meier survival curves, or cumulative incidence curves depending on the secondary endpoint of interest.

Patients that failed to be matched with a CTL will be compared to the patients that are matched to a CTL and infused by analyzing the recipient's age, gender, race, ethnicity, HLA type and infection type. In addition, overall survival at 6 and 12 months post CTL search initiation will be estimated based on the Kaplan-Meier product limit estimator. The purpose of this comparison is to provide information on a concurrent group of controls.

## 5.8 Data and Safety Monitoring Board (DSMB)

Interim analyses will be conducted at times coincident with regularly scheduled meetings of the NHLBI-appointed Data and Safety Monitoring Board (DSMB) at approximately six-month intervals. The DSMB Chair will be notified each time a grade 3-5 unexpected or grade 5 expected AE occurs. Monitoring of the key safety endpoint will be conducted as described above, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified and information will be supplied to the DSMB. Policies of the DSMB are described in the SCCT Manual of Procedures. The stopping guideline serve as a trigger for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment.

## APPENDIX A

### REFERENCES

1. Boeckh M, Nichols WG, Papanicolaou G et al. Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies. *Biol.Blood Marrow Transplant.* 2003;9:543-558.
2. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat.Rev.Cancer* 2004;4:757-768.
3. Curtis RE, Travis LB, Rowlings PA et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. *Blood* 1999;94:2208-2216.
4. Cohen JM, Cooper N, Chakrabarti S et al. EBV-related disease following haematopoietic stem cell transplantation with reduced intensity conditioning. *Leuk.Lymphoma* 2007;48:256-269.
5. Brunstein CG, Weisdorf DJ, DeFor T et al. Marked increased risk of Epstein-Barr virus-related complications with the addition of antithymocyte globulin to a nonmyeloablative conditioning prior to unrelated umbilical cord blood transplantation. *Blood* 2006;108:2874-2880.
6. Kuehnle I, Huls MH, Liu Z et al. CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hemopoietic stem-cell transplantation. *Blood* 2000;95:1502-1505.
7. Comoli P, Basso S, Zecca M et al. Preemptive therapy of EBV-related lymphoproliferative disease after pediatric haploidentical stem cell transplantation. *Am.J Transplant* 2007;7:1648-1655.
8. Myers GD, Krance RA, Weiss H et al. Adenovirus infection rates in pediatric recipients of alternate donor allogeneic bone marrow transplants receiving either antithymocyte globulin (ATG) or alemtuzumab (Campath). *Bone Marrow Transplant.* 2005;36:1001-1008.
9. Feuchtinger T, Lucke J, Hamprecht K et al. Detection of adenovirus-specific T cells in children with adenovirus infection after allogeneic stem cell transplantation. *Br J Haematol* 2005;128:503-509.
10. Myers GD, Bollard CM, Wu MF et al. Reconstitution of adenovirus-specific cell-mediated immunity in pediatric patients after hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2007;39:677-686.
11. Cwynarski K, Ainsworth J, Cobbold M et al. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. *Blood* 2001;97:1232-1240.
12. Gottschalk S, Rooney CM, Heslop HE. Post-Transplant Lymphoproliferative Disorders. *Annu.Rev.Med* 2005;56:29-44.

13. Walter EA, Greenberg PD, Gilbert MJ et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med* 1995;333:1038-1044.
14. Peggs KS, Verfuether S, Pizzey A et al. Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. *Lancet* 2003;362:1375-1377.
15. Micklethwaite K, Hansen A, Foster A et al. Ex vivo expansion and prophylactic infusion of CMV-pp65 peptide-specific cytotoxic T-lymphocytes following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2007;13:707-714.
16. Einsele H, Roosnek E, Rufer N et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood* 2002;99:3916-3922.
17. Cobbold M, Khan N, Pourgheysari B et al. Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. *J Exp.Med.* 2005;202:379-386.
18. Rooney CM, Smith CA, Ng C et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr virus-related lymphoproliferation. *Lancet* 1995;345:9-13.
19. Rooney CM, Smith CA, Ng CYC et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood* 1998;92:1549-1555.
20. Heslop HE, Ng CYC, Li C et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nature Medicine* 1996;2:551-555.
21. Gustafsson A, Levitsky V, Zou JZ et al. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. *Blood* 2000;95:807-814.
22. Gottschalk S, Ng CYC, Smith CA et al. An Epstein-Barr virus deletion mutant that causes fatal lymphoproliferative disease unresponsive to virus-specific T cell therapy. *Blood* 2001;97:835-843.
23. Feuchtinger T, Matthes-Martin S, Richard C et al. Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. *Br.J Haematol.* 2006;134:64-76.
24. Leen AM, Myers GD, Sili U et al. Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. *Nat.Med.* 2006;12:1160-1166.
25. Haque T, Wilkie GM, Taylor C et al. Treatment of Epstein-Barr-virus-positive post-transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. *Lancet* 2002;360:436-442.

26. Sun Q, Burton R, Reddy V, Lucas KG. Safety of allogeneic Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for patients with refractory EBV-related lymphoma. *Br J Haematol* 2002;118:799-808.
27. Haque T, Wilkie GM, Jones MM et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood* 2007;110:1123-1131.
28. Gandhi MK, Wilkie GM, Dua U et al. Immunity, homing and efficacy of allogeneic adoptive immunotherapy for posttransplant lymphoproliferative disorders. *Am.J.Transplant.* 2007;7:1293-1299.
29. Peggs K, Verfuether S, MacKinnon S. Induction of cytomegalovirus (CMV)-specific T-cell responses using dendritic cells pulsed with CMV antigen: a novel culture system free of live CMV virions. *Blood* 2001;97:994-1000.
30. Nichols WG, Corey L, Gooley T et al. Rising pp65 antigenemia during preemptive anticytomegalovirus therapy after allogeneic hematopoietic stem cell transplantation: risk factors, correlation with DNA load, and outcomes. *Blood* 2001;97:867-874.
31. Ljungman P, Deliliers GL, Platzbecker U et al. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. The Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Blood* 2001;97:388-392.
32. Przepiorka D, Weisdorf D, Martin P et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995;15:825-828.