CHARMS

Administration of Most Closely HLA-matched Multivirus-specific Cytotoxic T-Lymphocytes for the Treatment of EBV, CMV, Adenovirus, HHV6, and BK virus Infections post Allogeneic Stem Cell Transplant

PRINCIPAL INVESTIGATORS
Bilal Omer, MD

CO-INVESTIGATORS
Carl Allen, MD, PhD
Nabil Ahmed, MD
George Carrum, MD
Adrian Gee, PhD
Meenakshi Hegde, MD
Rammurti Kamble, MD
Robert Krance, MD
Hao Liu, PhD
Caridad Martinez, MD
Swati Naik, MD
Carlos Ramos, MD
Rayne Rouce, MD
Ghadir Sasa, MD
Premal Lulla, MD

IND Number: 15092

Texas Children's Hospital
6621 Fannin Street
Houston, TX 77030-2399

Houston Methodist Hospital
6565 Fannin Street
Houston, TX 77030-2707

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CHECKLIST FOR PATIENT ELIGIBILITY AND NECESSARY INFORMATION: **CHARMS**

PATIENT ID_____PATIENT NAME _____

YES	NO	VALUE/DATE	
Any "NO" answers will make a patient ineligible for study participation.			
			Received prior myeloablative or non-myeloablative allogeneic hematopoietic stem cell transplant using bone marrow, single or double cord blood, or PBSC
			Cells Administered as treatment for; Persistent or relapsed CMV, adenovirus, EBV, HHV6 or BK virus (single or multiple) infections despite standard therapy(defined in section 2.3.1) OR Progressive or persistent JC virus infection (defined in section 2.3.1) Patients with multiple CMV, EBV, Adenovirus, HHV6 and BK virus infections are eligible given that each infection is persistent despite standard therapy as defined above. Patients with multiple infections with one or more reactivation and one or more controlled infection are eligible to enroll.
			Steroids less or equal to 0.5 mg/kg/day prednisone (or equivalent)
			HgB>8.0
			Pulse oximetry of >90% on room air
			Available multivirus-specific T lymphocyte lines
			Negative pregnancy test (if applicable)
			Patient or parent/guardian capable of providing informed consent
Any "YES" answers will make a patient ineligible for study participation.			
			Received ATG, Campath or other T cell immunosuppressive monoclonal antibodies in the last 28 days of screening for enrollment
			Patients with other uncontrolled infections (see 2.3.2 for definitions)
			Patients who are less than 28 days removed from their allogeneic hematopoieteic stem cell transplant or who have received donor lymphocyte infusions (DLI) within 28 days.
			Evidence of active acute GVHD $\geq$ grade 2
			Active and uncontrolled relapse of malignancy

Signature of MD _____ **Date** _____

PROTOCOL SYNOPSIS

Administration of Most Closely HLA-Matched Multivirus-Specific Cytotoxic T-Lymphocytes for the Treatment of EBV, CMV, Adenovirus, HHV6, and BK virus Infections post Allogeneic Stem Cell Transplant

Study Design:

The primary purpose of the study is to evaluate whether most closely HLA-matched multivirus-specific T cell lines obtained from a bank of allogeneic virus-specific T cell lines (VSTs) have antiviral activity against five viruses: BK virus, HHV6, EBV, CMV and adenovirus.

Reconstitution of anti-viral immunity by donor-derived VSTs has shown promise in preventing and treating infections associated with CMV, EBV and adenovirus post-transplant. More recently, an ongoing phase I/II study at our institution indicates that this approach can be extended to include additional viruses including BK virus and HHV6. 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 partially HLA-matched allogeneic VSTs. A prior phase II study at our institution using trivirus-specific VSTs generated using monocytes and EBV-transformed B cells gene-modified with a clinical grade adenoviral vector expressing CMV-pp65 to activate and expand specific T cells showed the feasibility, safety and activity of this approach for the treatment of refractory CMV, EBV and Adenovirus infections. However, the production process was lengthy, requiring 8-12 weeks, with exposure to biohazards (B95.8 EBV viral strain and adenovector), while antigenic competition between different viral components precluded increasing the spectrum of specificity beyond these three viruses.

We have overcome these limitations and in the current trial, we will evaluate whether rapidly generated, allogeneic most closely HLA-matched multivirus-specific VSTs, activated using overlapping peptide libraries spanning immunogenic antigens from BK virus, HHV6, CMV, adenovirus and EBV will be safe and produce anti-viral effects in allogeneic HSCT recipients infected with one of more of the targeted viruses that are persistent despite conventional anti-viral therapy. The study agent will be assessed for safety (stopping rules defined) and antiviral activity.

Primary Objective:

The primary objective of this pilot study is to determine the feasibility and safety of administering partially HLA-matched VSTs specific for five virsues in HSCT recipients with persistent viral reactivations or

infections. Patients will be closely monitored for any possible association between VST infusion and GVHD.

Secondary Objectives: Secondary objectives are to determine: effects of partially HLA-matched VST infusion on viral load and viral reactivations within 12 months, reconstitution of antiviral immunity, persistence of infused partially HLA-matched VSTs and effects on viral clinical signs and symptoms. Additional endpoints include secondary graft failure, development of chronic GVHD, clinical response to VST infusions, effects of HLA matching with clinical response and overall survival.

Eligibility: Patients will be eligible following any type of allogeneic transplant if they have BK virus, HHV6, CMV, adenovirus and/or EBV infection persistent despite standard therapy (as defined in section 2.3). In addition patients with progressive or persistent JC virus infection (which is highly homologous to BK virus) may also be enrolled. If patients are receiving steroids for treatment of GVHD or for other reasons, dosage must have been tapered to 0.5 mg/kg/day 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.

Treatment Description: The treatment schedule is as follows:
Patients will receive 2 x 10^7partially HLA-matched VSTs/m^2 as a single infusion. In the rare case where insufficient banked cell product is available, a lower number of cells may be infused after discussion with the principal investigator, patient and/or guardian and the treatment team. If they have a partial response (as defined by a 50% fall in viral load) they are eligible to receive up to 4 additional doses from day 28 after the initial infusion and at 2 weekly intervals thereafter. The viral load of the virus (or viruses) that patients are initially treated for must be monitored by viral PCR. GVHD scores will be recorded at the intervals defined.

Accrual Objective: A maximum of 80 patients will receive at least one infusion of VSTs.

Accrual Period: The estimated accrual period is 4 years.

Study Duration: Patients will be followed for toxicity for 28 days, acute GVHD for 6 weeks; anti-viral responses for up to 12 months and long term follow up including chronic GVHD for 12 months following the final VST infusion.

STUDY SCHEMA

Aim: To determine whether partially HLA-matched VSTs are safe and have antiviral activity against EBV, CMV, Adenovirus, HHV6 and BK virus.

Inclusion Criteria	**Exclusion Criteria**
1) Received prior myeloablative or non-myeloablative allogeneic hematopoietic stem cell transplant using either bone marrow, single/double cord blood or PBSC 2)Cells administered as; a) Treatment of relapsed or persistent reactivation or infection for EBV, CMV, Adenovirus, HHV6 and/or BK virus despite standard therapy. Multiple infections are eligible to enroll. b) Early treatment for single or multiple infections with EBV, CMV, adenovirus, HHV6 and/or BK virus following treatment failure or unable to tolerate standard therapy c) Treatment of progressive or persistent JC virus infection 3) Steroids less or equal to 0.5 mg/kg/day prednisone 4) Hgb>8.0 5) Pulse oximetry of > 90% on room air 6) Available VSTs 7) Negative pregnancy test (if female of childbearing potential after reduced intensity conditioning) 8) Patient or parent/guardian capable of providing informed consent.	1) Received ATG, Campath or other T cell immunosuppressive monoclonal antibodies in the last 28 days. 2) Patients with other uncontrolled infections (see 2.3.2 for definitions) 3) Patients who are less than 28 days removed from their allogeneic hematopoieteic stem cell transplant or who have received donor lymphocyte infusions (DLI) within 28 days. 4) Evidence of GVHD $\geq$ grade 2 5) Active and uncontrolled relapse of malignancy

Up to 4 additional doses can be administered from day 28 after the initial infusion if a partial response is obtained and patient meets eligibility criteria for subsequent infusions. Subsequent doses should be at least 14 days apart.

Primary endpoints:
-Feasibility of finding a suitable line
-Safety including acute GVHD grades III-IV within 42 days of the last dose of VSTs or grades 3-5 non-hematological adverse events within 28 days of the last VST 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 4.X.

Secondary Endpoints
-Antiviral responses 42 days after the first dose of VSTs
-Effects of partially HLA-matched VSTs on viral loads
-Persistence of partially HLA-matched VSTs and effects on clinical signs/symptoms
-Reconstitution of antiviral immunity at 1, 2, 3, 4,6 weeks and 3 months
-Viral reactivations within 12 months
-Secondary graft failure at 30 days
-Chronic GVHD at 3 months, 6 months and 12 months
-Clinical response to VST infusions at 6 weeks and 3 months
-Effects of HLA matching
-Effects of choosing a line with confirmed antiviral activity through one or more shared alleles
-Overall survival at 3 months, 6 months and 12 months

1 BACKGROUND AND RATIONALE

1.1 Viral Infection Post Transplant

Viral infections are normally controlled by T-cell immunity. Thus, in the immunocompromised setting (e.g. the period of immune recovery after hematopoietic stem cell transplantation (HSCT)) viral infections are a major cause of morbidity and mortality. Risk for infection is dictated by the degree of tissue mismatch between donor and recipient, and the resultant degree of immunosuppression, and by the immune status of the donor. Reactivation of latent viruses such as cytomegalovirus (CMV), BK virus, Human Herpesvirus 6 (HHV6) and Epstein-Barr virus (EBV) are common and often cause symptomatic disease. Respiratory viruses such as adenovirus also frequently cause infection. Antiviral pharmacologic agents are only effective against some of these viruses; their use is costly, and 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 already been successfully used to target some of these viruses.

1.2 CMV

Cytomegalovirus (CMV) is a latent beta-herpesvirus 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.[1] Cell-mediated immunity is considered the most important factor in controlling CMV infection and CMV-specific $CD4^+$ and $CD8^+$ lymphocytes play an important role in immune protection from 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, but both have major side effects including neutropenia and nephrotoxocity.

1.3 Epstein-Barr Virus

Epstein-Barr virus (EBV) is a gamma-herpesvirus 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, which differ in their viral antigen expression patterns.[2] In immunocompromised hosts, outgrowth of B cells expressing Type 3 latency (expression of all seven latency associated proteins)may lead to the development of post-transplant lymphoproliferative disease (PTLD). The overall incidence of PTLD after HSCT is approximately 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).[3-5]PTLD is highly susceptible to control by virus-specific T cells[6;7].

Few small molecule drugs have any effect on B cells already transformed by EBV and while nucleoside analogs including ganciclovir inhibit the viral explicative cycle their clinical benefit is questionable at best. Conventional chemotherapy, while effective in some cases is associated with significant toxicity with high treatment related mortality in the HSCT patient population. One

option for prophylaxis and treatment of PTLD after HSCT is rituximab, a monoclonal antibody against the B cell phenotypic antigen, CD20.[5;8;9]However, not all patients respond and rituximab depletes normal B-cells for more than 6 months, which can be problematic in a patient population that is already immunosuppressed.

1.4 Adenovirus

Adenovirus is a non-enveloped lytic DNA virus. Humans are susceptible to infection with 51 serotypes forming six distinct adenovirus species (A to F), which differ in their tissue specificity and virulence. Although causing acute infections, adenoviruses may persist for many months after resolution of disease and therefore are frequently carried undetected into the transplant by donor or recipient. While acute infection is rarely fatal in healthy adults, it is a 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.[10] Several reports have shown that clearance of adenovirus infection is associated with detection of adenovirus-specific T cells[11;12] and recovery is significantly delayed in recipients of matched unrelated donor and haploidentical transplant who receive intensive immunosuppression such as Campath.[12] The most frequently used drug for disease treatment is Cidofovir[13], but the associated nephrotoxicity is a major concern. Moreover, in the absence of prospective, randomized, controlled trials, the efficacy of the drug is uncertain.

1.5 BK virus

Human polyomavirus type I, commonly called BK virus (BKV), infects 50% to 90% of humans worldwide before the age of 10 years, without known symptoms or signs. Polyomavirus disease in HSCT patients most often corresponds to secondary BKV replication with impaired polyomavirus-specific cellular immunity. Urinary shedding of BKV occurs in 60-80% of HSCT recipients[14]. The major disease linked to high-level polyomavirus replication is BKV-associated hemorrhagic cystitis (PVHC), which affects 5-15% of HSCT recipients at 3-6 weeks post-transplant. BKV viruria reaching high viral loads of>10^7 genome equivalents/mL is observed in 20-80% of HSCT patients, but less than one fifth of HSCT recipients develop PVHC.

No clear standard treatment is defined -cidofovir[15] has been administered intravenously in a low dose (i.e. up to 1 mg/kg 3 times weekly, without probenecid) or a high dose (i.e., 5 mg/kg per week with probenecid) to HSCT patients with BK infections but no randomized trials are available proving its clinical efficacy. There is no evidence to support the use of cidofovir for pre-emptive treatment of asymptomatic HSCT patients who develop BK viruria or viremia. Ciprofloxacin can inhibit BKV replication in tissue culture, but only scant data exists regarding its efficacy after HSCT[16], and is limited mostly to the prophylactic setting. In small trials leflunomide had some activity against BK virus[17]; therefore we will consider this agent an acceptable alternative to cidofovir, given the absence of a clear first line option.

1.6 HHV6

Nearly all children are infected with Human herpesvirus 6 (HHV6) by 3 years of age and the viruses causes the classic childhood illness roseola. After primary infection, HHV-6 remains latent in lymphocytes and monocytes unless the immune system is compromised, at which time the virus may reactivate[18;19.] HHV6 is increasingly recognized as an important cause of morbidity and mortality in patients after HSCT, with viremia occurring in 40 to 60% of transplant recipients.

HHV-6 infection generally occurs earlier than CMV infection. The median time to onset in accumulated studies has been shown to be 22 days and up to 70% of infections occur within the first 6 weeks of transplantation.

This is an immunomodulatory and immunosuppressive virus that has been shown to facilitate superinfection with opportunistic infections, particularly fungal infections. It also has been shown to be a cofactor in CMV disease and concurrent infection with both these herpesviruses as opposed to CMV alone is strongly associated with CMV hepatitis and CMV viral syndrome.

HHV6 reactivation is common during the early period post allogeneic HSCT transplant, with viremia occurring in approximately 40-60% of patients. In bone marrow transplant recipients, there is clearly an association between HHV6, marrow suppression and delayed engraftment and HHV6 very likely is a contributor to graft rejection. There are emerging data to show that it is an important cause of encephalitis in bone marrow transplant recipients. The presentation of HHV-6 encephalitis in some ways is nonspecific. The most common clinical manifestation is mental status changes that can range anywhere from lethargy to more severe mental status depression. Seizures, headaches, speech disturbances, and fever have each been detected in about a quarter of the patients. Focal neurologic findings in HHV-6 encephalitis are somewhat unusual.

Ganciclovir, cidofovir, and foscarnet have variable in vitro activity against HHV-6, and may have a role in treating HHV6-associated disease, however response is not consistent and the associated nephrotoxicity and myelosuppression remains a major concern.[14]

1.7 Treatment of Patients with JC virus

Human polyomavirus type I, commonly called BK virus (BKV), and the highly homologous human polyomavirus type II, commonly called JC virus (JCV), infect 50-90% of humans worldwide before the age of 10 years, without known symptoms or signs. Reactivation of JCV can occur with immunodeficiency, as occurs after HSCT.

One of the most feared complications is the rare, but generally fatal progressive multifocal leukoencephalopathy (PML), which is associated with the destruction of oligodendrocytes by JCV. Therapeutic options are limited and no recognized standard therapy exists. Isolated case reports had suggested a possible benefit of mefloquine, however a recent open-label, randomized, parallel-group, proof-of-concept study found no evidence of anti JCV activity by mefloquine in PML patients[46].

1.8 Adoptive Immunotherapy with virus-specific T cells

Since recovery of virus-specific T cells is clearly associated with protection from infection with each of these viruses,[11;20;21] 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 (APCs) expressing viral antigens have been evaluated in clinical trials to prevent and treat viral infections in immunocompromised hosts.[22-26]This approach eliminates alloreactive T cells.

There are several considerations in developing protocols for generating VSTs 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 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.

1.8.1 Therapy with VSTs specific for CMV

In the first study evaluating whether adoptively-transferred T cells could reconstitute anti-viral immunity, CMV-specific T-cell clones were derived from sibling donors after stimulation with autologous fibroblasts pulsed with CMV.[25] There were no adverse effects and CMV-specific immune responses were reconstituted, with none of the patients developing CMV disease or late recurrence. In another prophylaxis study, Peggs et al., generated CMV-specific CD4+ and CD8+ 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[26]. Small doses of VSTs were able to reconstitute immunity with considerable *in vivo* expansion of CMV-specific VSTs. To avoid the use of live CMV during T cell manufacture, a more recent study stimulated VSTs with dendritic cells pulsed with the HLA-A2 restricted peptide NLV derived from the cytomegalovirus-pp65 protein.[27] While this approach also appeared effective, a concern is the restricted specificity of the infused VSTs, since targeting a single epitope may allow escape variants and limits the study to patients who are HLA A2-positive.

CMV-specific VSTs have also been used therapeutically in patients with CMV infection that has persisted or recurred despite prolonged antiviral medication.[28] The results were encouraging, with suppression of viral reactivation in 6 of 7 subjects. In this study, the source of antigen was a CMV lysate, which has the advantage of producing a broad immune response but which is unsuitable for Phase III studies because of the risk of infection from live virus in the lysate.

The VST cell therapies described above all employed methods of T cell production that require prolonged periods of activation and expansion in specialized GMP facilities with significant regulatory support. These requirements reduce the practicality of adoptive immunotherapy since VST lines must be made long in advance of disease and few centers have the facilities or infrastructure required for this type of cell processing. To overcome these problems, two direct T cell selection approaches have been used to isolate CMV-specific T cells from peripheral blood without the requirement for ex vivo expansion. The first approach is the interferon (IFN)γ-capture assay, which isolates IFNγ-secreting T cells after antigen exposure. Another approach is using a MHC-peptide-multimer-construct, e.g. streptamer selection.

1.8.1.1 IFNγ-capture of CMV-specific T cells

Feuchtinger and colleagues used IFNγ-captured CMV-specific T cells to treat chemorefractory CMV disease or reactivation after allogeneic HSCT. After short-term stimulation and selection a mean of 21.3×10^3 CD3+ cells per kg was infused to 18 patients, without acute side effects and only a single case of GVHD. In 15 of 18 infused patients clearance of CMV viremia or a significant reduction (>1 log) of viral load was associated with the adoptive transfer. Peggs et al also reported the clinical results associated with the adoptive transfer of IFNγ-captured CMV-specific T cells. In this prophylaxis/preemptive treatment study, pp65 protein or peptide pools were used to stimulate T cells. Post-selection a median of 2840 CD4+ and 630 CD8+ CMV-specific T cells/kg were infused early (median day 35) post-transplant and expansion of both populations was detected in

vivo. However, unlike the Feuchtinger study, acute GVHD was observed in 8 of 18 patients, although this was mild in most cases with only 2 patients requiring systemic steroid therapy.

1.8.1.2 Multimer-based isolation of CMV-specific T cells

Multimer techniques such as tetramers have also been developed for the direct selection of antigen-specific T cells. For example Cobbold and colleagues performed a clinical trial using tetramer selection of CMV peptide-specific T cells directly from peripheral blood. Cells were infused to patients within 4 hours of selection. Although exclusively CD8+ T cells were infused, they expanded by several logs after infusion, clearing infection in 8/9 cases.[29]More recently streptamers have been used to clinically select distinct T cell populations for adoptive transfer. Streptamers, because of the biotin-streptavidin interaction, detach from T cells over time and are thus not considered to be drugs, but rather adjuvant. This technical feature distinguishes streptamers from other multimers and facilitates their clinical use. Schmitt and colleagues recently reported the first clinical experience of streptamer-selected CMV-specific T cells[30]. After a single infusion of 2.2×10^5 HLA-B7+/CMV pp65-specific CD8+ T cells/kg (purity of 97%), the frequency of CMV-specific T cells increased dramatically in vivo from 0% prior to infusion to a maximum of 27.1% of all T cells. These T cells were confirmed to be donor in origin by analysis of donor chimerism through single-tandem repeats, T cell receptor excision circles, and Vβ-chain typing. Clinically, the T cell infusion resulted in CMV clearance without alloreactivity.
Though both approaches have been associated with clinical benefit, the multimer strategy is limited by the HLA restriction of antigen recognition, the availability of clinical grade multimers and lack of HLA class II multimers. Additionally, both the multimer and IFNγ approaches require large numbers of starting peripheral blood material, which may not always be readily available (e.g. from an unrelated donor).

1.8.2 Therapy with VSTs specific for EBV

EBV-transformed lymphoblastoid cell lines (LCLs) generated by infecting peripheral blood B cells with a laboratory strain of EBV can be readily prepared from any donor, they have been used as APCs in clinical studies evaluating EBV specific VSTs.[6;7;9;31;32]We have shown that adoptively transferred EBV-specific VSTs can survive for >10 years, expand up to 2-4 logs after infusion, and reduce the high virus load that is observed in about 20% of patients.[6;7;24]In a recent review of three studies targeting high-risk patient populations, none of 101 patients who received EBV VSTs as prophylaxis developed PTLD.[24]Of 13 patients with active PTLD at the time of infusion, donor-derived EBV-specific VST lines induced remission in 11,[24] while in one of the non-responders, tumor virus had deleted the immunodominant epitopes in one of the viral antigens that were the targets of the infused effector T cells.[33]Numerous other studies have confirmed the activity of ex vivo expanded EBV-specific VSTs post transplant.[9;32]

Rapid selection techniques have also been used to directly isolate EBV-specific populations for infusion. Indeed, Moosmann and colleagues isolated IFNγ secreting cells following exposure to 23 class I and II peptides derived from 11 EBV antigens, which were infused into 6 patients with EBV-PTLD[34]. While three patients responded, three with more advanced disease progressed. Whether these patients would have responded had they received higher numbers of effector T-cells remains an important question for future development. Icheva and colleagues specifically targeted EBNA1 and pulsed APCs with either whole EBNA1 protein or EBNA1 overlapping peptide pools and then selected responding T cells by IFNγ capture. Ten patients with PTLD were treated and 7 had clinical responses. No significant toxicities were seen in these studies. Finally, Uhlin and colleagues isolated, using multimers, HLA A2-restricted T cells specific for epitopes in

two EBV antigens from a haploidentical parent to treat EBV PTLD in a cord blood transplant recipient[35]. Post-infusion a small number of these directly selected cells expanded in vivo and produced a complete clinical response. At 12 months after transplant the EBV-PTLD recurred, but a second multimer selection and infusion induced a second response.

1.8.3 Therapy with VSTs specific 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 IFNγ-secreting cells.[36] 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.[36]

1.8.4 Trivirus-Specific Donor-Derived T Cells

Most studies with VSTs have only targeted single viruses. To broaden the specificity of single VST lines to include three of the most common viral pathogens of stem cell recipients we previously generated virus-specific VSTs using a recombinant adenoviral vector encoding the CMV antigen pp65.[23] This method reliably produced VSTs with cytotoxic function specific for all three viruses in a process requiring 8-12 weeks for manufacture. When we infused these cells to 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.[23] A follow-up study in which the frequency of adenovirus-specific T cells was increased in the infused VSTs produced similar results, thus highlighting the importance of endogenous antigen to promote the expansion of infused T cells in vivo.[22] Nevertheless, all patients in both clinical trials 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.[23]

More recently, our group has sought to simplify and accelerate T-cell expansion with minimal cell handling, while ensuring that T-cell specificity and function is maintained. The first step was to replace EBV-LCLs and adenovirus vectors as a source of antigen and APC. To eliminate the six weeks required for EBV-LCL manufacture, we investigated two alternate sources of biohazard-free antigen sources (plasmids and overlapping peptide libraries) as well as dendritic cells (DCs) or APCs present in peripheral blood to activate virus-specific T cells. To first assess whether plasmids could substitute for EBV-LCL/adenovectors we generate clinical grade plasmids encoding immunogenic EBV (EBNA1, LMP2, BZLF1), CMV (IE1, pp65) and adenovirus (Hexon, Penton) antigens and used DCs nucleofected with these plasmids to generate trivirus-specific T cells, which were expanded in vivo in the presence of the cytokines IL4+7 in a G-Rex device for 10 days before being cryopreserved for clinical use. To test the in vivo activity of these cells we have recently completed a phase I/II treatment study, achieving a response rate of 80% in patients with single or multiple active infections[37], without adverse effects. Thus, VSTs recognizing multiple antigens from 3 viruses can produce clinically relevant effects in vivo.

1.8.5 Multivirus specific Donor-Derived VSTs targeting BK, HHV6, Adv, EBV and CMV

BK virus and HHV6 are increasingly recognized as important contributors to morbidity and mortality in patients after HSCT and therefore including these two viruses in addition to adenovirus, EBV and CMV in a multivirus cellular immunotherapy strategy that targets all five of these viruses significantly increases clinical utility.

1.8.5.1 Generation of multivirus-directed VSTs by direct stimulation of PBMCs with clinical grade pepmixes.

We have developed a 10-day GMP-compliant T cell production process to generate T cell lines with simultaneous specificity for 5 viruses (Adv, EBV, CMV, BK and HHV6). To activate PBMCs we expose PBMCs directly to a mastermix of pepmixes (peptide libraries of 15mer peptides overlapping by 11 amino acids) spanning immunogenic antigens derived from each virus; - adenovirus (Hexon and Penton), CMV (IE1 and pp65), EBV (LMP2, EBNA1, BZLF1), BK virus (VP1 and large T), and HHV6 (U90,U11 and U14). After activation cells are transferred to a G-Rex10 device for 9-11 days and cultured in media containing IL4 and IL7 to achieve maximal expansion of antigen-specific T cells without carry-over of alloreactive T cells[38]. From starting cell numbers of $15x10^6$ PBMCs/G-Rex we were reproducibly able to produce 1-1.2 x 10^8 antigen-specific VSTs in the stated timeframe. These multivirus VST lines are polyclonal and polyfunctional, with activity against the stimulating viruses detectable in both the CD4+ and the CD8+ T cell fractions[38].

1.8.5.2 Clinical activity of donor-derived multivirus-directed VSTs

We evaluated the clinical utility of multivirusVSTs in recipients of matched related, matched unrelated, or haploidentical donor transplants. To date, 35 clinical-grade multivirus-directedVSTs have been generated from donor PBMCs.These lines were polyclonal, comprising both CD4+ (57±5%) and CD8+ (35±5%) cells and retained expression of the memory markers CD45RO+CD62L+ (58±8%). Their specificity was dependent on the prior viral exposure of the cell donor; 32/35 lines had activity against Adv, 20/35 against CMV, 26/35 against EBV, 18/35 against BK and 21/35 against HHV-6. None of the lines reacted against recipient PHA blasts – indicating lack of alloreactive potential in these rapidly generated lines.

We administered these multivirus-specific donor-derived VSTs to 10 allogeneic HSCT recipients in a dose escalation study; 4 on DL1 ($5x10^6/m^2$), 4 on DL2 ($1x10^7/m^2$) and 2 on DL3 ($2x10^7/m^2$). There were no immediate infusion toxicities, and no de novo acute GvHD, demonstrating the in vivo safety of these mVST. Three patients received the cells as viral prophylaxis (days 38-43 post-HSCT) and none developed viral infections at up to 3 months post-treatment. The other 7 patients received the cells as treatment for one or more active infections between days 59-139 post-HSCT. Based on viral load measurements by day 42 post-infusion, the VSTs were successful in controlling active infections with CMV (1 complete (CR) and 1 partial response (PR)), EBV (2 CRs, including a case of frank PTLD); Adv (1 CR); HHV6 (1 CR); and BK (3 CR, 1 PR, 1NR). Of note, 3 BK virus responders had tissue disease with severe hemorrhagic cystitis and all had marked improvement or disappearance of hematuria following infusion. One patient subsequently had an episode of transient but severe bladder pain in association with inflammation seen on cytoscopy coincident with a 6 log fall in urine BK viral load. Our only non-responder was a patient with BK infection whose line lacked activity for this virus, likely reflecting the serostatus of the donor. In addition, 3 patients subsequently reactivated other viruses than those for which they were initially treated, but all cleared these infections by week 12, without the requirement for additional cell infusions (CMV: 1CR; EBV: 1CR; BK: 1CR; HHV6: 1CR). Finally, 1 patient received multivirus specific VSTs under a single patient protocol as an emergency treatment for widespread and bulky rituximab-resistant EBV-PTLD. Post VST treatment there was an immediate decline in the patient's EBV viral load with complete and sustained resolution of PTLD, coincident with an increase in circulating EBV-specific T cells. However, the profound anti-tumor activity mediated by the rapidly-expanding EBV-directed T cells also produced a transient

systemic inflammatory response syndrome, which was controlled with steroids and anti-TNFR antibody, with no long term adverse effects.

Thus, infusion of donor-derived, multivirus specific VSTs generated with clinical grade pepmixes and infused either prophylactically or as treatment for one or more viral infections has been safe and is associated with the appearance of virus-reactive T cells in peripheral blood that have been able to control infection with all 5 targeted viruses.

1.9 Limitations of donor-derived virus specific VSTs

Despite the successful use of donor-derived multivirus-directed VSTs in these trials, there are some limitations to this approach. One problem is that virus-specific VST lines can only be generated from donors who had previously been exposed to a virus and therefore cannot be generated from seronegative donors or cord blood. There is increasing evidence over the last years that the use of younger donors is associated with improved engraftment, decreased relapse rates and improvements in overall and event-free survival in HSCT patients[39]. However younger donors are also more likely to be seronegative for several of the targeted virus, which along with the expanding use of cord blood transplants increases the number of patients for which donor-derived VSTs cannot be generated. Another problem is that despite the significant decrease in the amount of time it takes to manufacture virus-specific VSTs with our pepmix-based method (decrease from ~3 months to just 10 days), this time frame is still too long for patients with more fulminant viral disease.

1.10 Most closely matched allogeneic virus-specific VSTs

An alternative approach that bypasses the need to grow VSTs for individual patients is to bank closely HLA-matched allogeneic VSTs 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 studies have demonstrated the feasibility of this approach and reported clinical responses in the patients with EBV lymphoma arising after HSCT or solid organ transplant.[40;41]In the first and largest multicenter study, Haque et al used banked polyclonal EBV-specific T-cell lines to treat EBV-PTLD after HSCT or solid organ transplantation and reported an overall response rate of 52% at 6 months. Similar results have been reported from Memorial Sloan Kettering with four of five PTLD patients achieving CR in response to third party EBV-specific T-cells. Of note none of these studies report an increased risk of GVHD.

More recently our group has applied this approach to treat patients with refractory CMV, Adenovirus and EBV infections post allogeneic HSCT. Lines were generated using adenovector-pp65 transduced monocytes and EBV-LCL and were either retained from our prior donor-specific clinical study or were newly generated from donors with known antiviral activity, including HLA homozygous donors identified by the National Marrow Donor Program. A total of 32 lines were produced and characterized, 18 of which were administered to the 50 study patients. The selection of lines for infusion was based on the specificity of the line for the target virus through a shared HLA allele, as well as the overall level of HLA match.

This study was open to allogeneic HSCT recipients with CMV, AdV, or EBV infection that had persisted for at least 7 days despite standard therapy. Patients who had a suitable VST line

received an intravenous infusion of up to 2 × 10^7VSTs/m^2 and were eligible for additional infusions at intervals of at least 2 weeks, in the event of a partial response (PR). Of the 50 patients who received VST infusions 23 received VSTs for persistent CMV, 18 for persistent adenovirus, and 9 for refractory EBV-PTLD. Five of the 50 treated patients withdrew from the study or died of their underlying disease.

From the bank of 32 VST lines a suitable line was identified for 90% of the screened patients within 24 hours. Of the 50 patients who were treated with these VSTs 74.0% had a CR or PR (73.9% for CMV, 77.8% for AdV, and 66.7% for EBV), including responses in 6 of 9 patients with refractory EBV-PTLD, and most of these responses (89%) were durable. By contrast 8 patients in the study for whom a line was not available and who continued with "standard therapy" demonstrated a 13% response rate, and 6 (75%) died of viral disease.

1.11Risks of Administering Virus specific VSTs

1.11.1 Risk of Administering Donor-derived VSTs

We recently reviewed the infusion-related adverse events (AE) following administration of ex vivo-expanded T cell products (antigen-specific cytotoxic T lymphocytes, allodepleted T cells, and genetically modified T cells) on investigational new drug (IND) studies in our center[42]. From 1998 to 2008, we infused a total of 381 T cell products to 180 recipients, enrolled on 18 studies, receiving T cells targeting malignancies or post-transplant viral infections. There were no grade 3-4 infusion reactions during initial monitoring or 24-h follow-up. Twenty-four mild (grade 1-2) AE occurred in 21 infusions either during or immediately following infusion (up to 6hrs), most commonly nausea and vomiting (41.6%), probably because of the dimethyl sulfoxidecryoprotectant, and hypotension (20.8%), attributable to diphenhydramine pre-medication. Twenty-two additional non-severe events were reported within 24 h of infusion, most commonly culture-negative fever, chills and nausea. An increased risk of AEs was associated with age [incidence rate ratio (IRR) 0.98; 95% confidence interval (CI) 0.96-1.00, P = 0.05], while an increased risk of immediate infusion-related events was higher in patients reporting allergies (IRR 2.72, 95% CI 1.00-7.40, P = 0.05); sex, disease type and T cell source (allogeneic or autologous) had no effect on frequency of adverse events[42].

Focusing specifically on VSTs, over 125 patients have been infused with EBV or multivirus VSTs in the donor-specific setting. The vast majority (123) occurred with no or minimal toxicity consisting mainly of localized swelling at sites of responsive disease. Two patients infused with donor-specific products developed syndromes consistent with systemic inflammatory response syndrome (SIRS). The first patient was infused with EBV VSTs as treatment for bulky disease including extensive pharyngeal disease. He had a vigorous inflammatory response with an immune infiltrate of genetically marked cells apparent on follow up biopsy. This response produced progressive airway obstruction and mucosal sloughing, ultimately requiring mechanical ventilation. He also had reversible cardiac impairment and fevers during this response. This patient subsequently made a full recovery and remains well more than 10 years later. He was treated in 1996 when cytokine panels were not available so we cannot conclude that this was a SIRS, but it seems likely. The second patient was infused with multivirus VSTs as treatment for rituximab-resistant EBV-PTLD, with bulky disease. Approximately two weeks after receiving the VSTs she developed a fever, became tachycardic requiring fluid boluses and eventually required transfer to ICU with fever, tachycardia and hypotension for inotropic support. She required Dopamine and Norepinephrine to maintain her blood pressure and appeared to be in warm shock. The differential diagnosis of her treating physicians was sepsis or SIRS associated with VST

therapy. She also had a diffuse erythematous rash consistent with drug reaction, infection or GVHD. As her blood cultures were negative and she continued to require inotropic support, was given empiric etanercept as well as methylprednisolone (1 mg/kg daily x2) and her symptoms resolved within a few hours. She remains well.

In other reported studies none of the patients treated by Walter et al. with CMV VST developed GVHD[25]. In the cohort of patients treated by Peggs, 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 VST infusion.[43]

1.11.2 Risk of 3rd party VSTs

In a study at Memorial Sloan Kettering 5 patients received a median of 5 doses of third party virus specific VSTs for EBV after HSCT for EBV LPD, most at $1x10^6$ EBV-VST/kg/infusion, and all infusions were well tolerated[44]. Updated data from the study was reported recently at the American Society of Hematology meeting 2012, with now 10 patients after HSCT enrolled in this study and among the 10 patients one patient developed mild skin GVHD after infusion with 3rd party EBV-VSTs, but tolerated a subsequent infusion of EBV-VSTs from an alternate 3rd party donor, with no other incidences of GVHD reported.

In our study of third party VSTs specific for three viruses (AdV, CMV and EBV) a total of 50 patients were infused with third party banked VSTs. All of the infusions were well tolerated. There were no immediate adverse effects, and despite the HLA disparity between the VSTs and recipients, de novo GVHD occurred in only 2 patients (grade I in each case). In the 8 patients in whom acute GVHD developed within 45 days of the first infusion (grade I in 6 patients, grade II in 1 patient, and grade III in 1 patient), 6 had a history of GVHD prior to receiving the VSTs. An additional patient had a flare of chronic skin GVHD. Two patients experienced transplant-associated microangiopathy, a complication that occurs in up to 10% of HSCT recipients, particularly in those receiving sirolimus[45], as were both of our patients. Only 1 patient had secondary graft failure, concomitant with leukemic relapse.

In order to minimize the risk of infusing a VST product with alloreactive potential in our studies of donor-derived VSTs we have screened 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 the possibility of alloreactivity 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 of donor origin 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 criterion. We will, however, take several additional precautions to minimize the risk of GVHD. First, any patient with pre-existing GVHD of ≥ Grade 2 will be excluded from the study. Second, we will administer VSTs at a dose of $2x10^7$cells/m^2. This is a much smaller number of T cells than administered at the time of an unmanipulated marrow infusion.

1.11.3 Cytokine Release Syndrome

There have been several reported SAEs associated with cytokine release syndrome (CRS) in patients who received T cells[46] or bispecific T-cell engagers[47]. The majority of CRS have been reported after the infusion of CAR T cells[48-50], but CRS can also occur after the infusion of conventional antigen-specific T cells[51] or tumor infiltrating lymphocytes[52]. Patients will be monitored closely as per study calendar and assessed for evidence of incipient CRS (onset of fever, malaise and dyspnea) and treated promptly. Management of CRS will follow published guidelines[46,53], and is described in more detail in SOP F 05.11.XX and includes treatment options based on the clinical severity of the symptoms, such as oxygen, inotropic agents, IL-6 receptor antibody (4-8 mg/kg),TNF-α antibody (5-10 mg/kg), and/or steroids (1-2 mg/kg/day of methylprednisolone or equivalent).

1.12 Treatment of patients with JC Virus

Recently the first use of ex vivo expanded polyomavirus-specific CTLs has been reported with successful control of JCV-induced progressive multifocal leukoencephalopathy post-HSCT[54,]. Balduzzi and colleagues treated an HSCT patient with resistant JCV-associated progressive multifocal leukoencephalopathy (PML) using donor-derived JCV-specific CTLs, which were generated in vitro after stimulation with 15-mer peptides derived from the BK virus VP1 and large T viral proteins[55]. After adoptive CTL infusion, virus-specific cytotoxic cells were detected in the peripheral blood, and coincided with JCV-DNA clearance in the cerebrospinal fluid. This case suggests that adoptive transfer of JCV-targeted CTLs may contribute to restore JCV-specific immune competence and control PML in transplanted patients. Given these promising results in the context of a complete lack of proven antiviral medications effective against JC virus and the potentially devastating complications associated with JC virus, patients with persistent JC virus infection will be eligible for this study.

2 STUDY DESIGN

2.1 Primary Objective

This trial is designed to evaluate the feasibility andsafety of partially HLA-matched VSTs specific for five viruses in HSCT patients with persistent EBV, CMV, adenovirus, BK and/or HHV6 infections.

2.2 Rationale for Study Design

The primary purpose of this pilot study is to assess the safety of administering partially HLA-matched VSTs in transplant patients with BK virus, HHV6, EBV, CMV, and/or adenovirus infection. We have elected to use a dose of 2 x 10^7VSTs/m^2, which has been shown to be safe and have clinical activity in a Phase I study using the same T cell product in the donor-specific setting. Because the persistence of adoptively transferred cells may be shorter using a partially HLA-matched banked VST product, we have included an 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, with expected antiviral activity against the infecting

virus through this single shared allele, although preference will be given to lines matching at the most loci where anti-viral activity through a shared allele has been confirmed.

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 or BK virus, although there are reports of cidofovir having activity. We have therefore chosen to use cidofovir as standard therapy for both these infections although this requirement would be waived if the subject could not tolerate this agent due to nephrotoxicity. Ganciclovir, cidofovir, and foscarnet have variable in vitro activity against HHV-6, and may have a role in treating HHV-6-associated disease. Hence standard therapy will include treatment with one or more of these agents.

2.3 Eligibility

2.3.1 Inclusion Criteria

For Initial VSTs and subsequent infusions: patients will be eligible following any type of allogeneic transplant (*as defined 2.3.1.1*) if they have CMV, adenovirus, EBV, BK virus and/or HHV6 infection/disease persistent or recurrent despite 14 days of standard therapy (*as defined 2.3.1.2*) OR after failure of treatment (*as defined 2.3.1.2*) after 7 days of standard therapy OR if unable to tolerate standard therapy. Patients with persistent JC virus infection will be eligible as well.

1. Prior myeloablative or non-myeloablative allogeneic hematopoietic stem cell transplant using either bone marrow or peripheral blood stem cells or single or double cord blood.

2. Treatment of the following persistent or relapsed infections despite standard therapy;

   a. CMV: Treatment of persistent or relapsed CMV disease or infection after standard therapy. For CMV infection, standard therapy is defined as antiviral therapy with ganciclovir, foscarnet or cidofovir.[56,57]

      i. 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 broncheoalveolar lavage fluid in the presence of new or changing pulmonary infiltrates or changes consistent with CMV retinitis on ophthalmologic examination.
      ii. CMV infection: defined as the presence of CMV positivity as detected by PCR or pp65 antigenemia or culture from ONE site such as stool or blood or urine or nasopharynx.

      Failure of antiviral therapy: 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 pp65 antigenemia after 7 days of antiviral therapy.

   b.Adenovirus: Treatment of persistent adenovirus infection or disease despite standard therapy. Standard therapy is defined as antiviral therapy with cidofovir[13] or an alternative antiviral agent if patient will not tolerate cidofovir therapy because of poor renal function.

i. Adenovirus infection: defined as the presence of adenoviral positivity as detected by PCR or culture from ONE site such as stool or blood or urine or nasopharynx.
ii. Adenovirus disease: defined as the presence of adenoviral positivity as detected by PCR, DFA or culture from two or more sites such as stool or blood or urine or nasopharynx.
iii. Failure of therapy: 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) after 7 days of antiviral therapy.

c. EBV: For treatment of persistent EBV infection despite standard therapy. For EBV infection, standard therapy is defined as rituximab given at 375mg/m^2 in patients for 1-4 doses with a CD20+ve tumor.[58]

i. EBV infection: 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/or elevated EBV viral load in peripheral blood.
ii. Failure of therapy is defined as
    1. Increase or less than 50% response at sites of disease for EBV lymphoma OR
    2. Increase or a fall of less than 50% in EBV viral load in peripheral blood or any site of disease after 1st dose of rituximab.

d. BK virus: Treatment of persistent BK virus infection or BK virus disease despite antiviral treatment with cidofovir or leflunomide. No clear standard treatment is defined (section 1.1.5). Cidofovir[15]has been administered in low doses as well as high doses to HSCT patients with BK infections but no randomized trials are available proving its clinical efficacy. In small trials leflunomide had activity against BK virus[17], therefore we will consider this agent an acceptable alternative to cidofovir, given the absence of a clear first line option.

i. BK virus infection is defined as the presence of BK virus positivity as detected by PCR or culture in one site such as blood or urine.

ii. BK virus disease is defined as the presence of BK virus detectable by culture or PCR in blood or urine or other body fluids and symptoms of disease including, but not limited to persistent microscopic or macroscopic hematuria or detectable BK virus in more than one site.

iii. Failure of therapy 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) or worsening hematuria after 7 days of antiviral therapy.

e. HHV6: Treatment of persistent HHV6 infection or disease despite antiviral treatment with ganciclovir, cidofovir and foscarnet. No clear standard treatment is defined.

Ganciclovir, cidofovir and foscarnet all have variable *in vitro* activity against HHV-6, and may have a role in treating HHV-6-associated disease – therefore antiviral treatment with one or more of these agents will we acceptable initial therapy.

i. HHV6 virus infection is defined as the presence of elevated HHV-6 levels as detected by PCR or positive culture in one site such as CSF or blood.

ii. HHV6 disease is defined as defined as the presence of HHV6 detectable by culture or PCR in one or more sites such as blood or CSF and symptoms of disease including symptoms of HHV6 encephalitis OR detectable HHV6 by PCR or culture in more than one site.

iii. Failure of therapy 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) after 7 days of antiviral therapy.

f. JC virus: Treatment of progressive or persistent JC virus infection or disease without suitable alternative treatment option. Pepmixes specific for antigens on adenovirus, EBV, CMV, HHV6 and BK virus are used to generate our multivirus-specific VSTs. No pepmix specific for the rare JC virus is used for generation of these CTLs, however given the high homology (>90%) between JC and BK and the fact that BK virus-specific T cells targeting VP1 and Large T (as targeted in our multivirus VSTs) have been administered to treat JCV-PML, resulting in viral clearance from the cerebrospinal fluid it is likely that our VSTs are efficacious against JC virus. Given the current lack of treatment options for JC virus infection or reactivation after HSCT and the risk of progression to JML, which is almost uniformly fatal, and the apparent activity of BK virus-directed T cells against JC virus infected cells, we propose including patients with progressive or persistent JC virus on this study, unless a suitable alternative therapy is available.

i. JC virus infection is defined as the presence of elevated JC virus levels as detected by PCR or positive culture in one site such as CSF or blood.

ii. JC virus disease is defined as defined as the presence of JC virus detectable by culture or PCR in one or more sites such as blood or CSF and symptoms of disease including symptoms of PML OR detectable JC virus by PCR or culture in more than one site.

3. Patients with multiple CMV, EBV, Adenovirus, HHV6 and BK virus infections are eligible given that each infection is persistent despite standard therapy as defined above. Patients with multiple infections with one or more reactivation and one or more controlled infection are eligible to enroll.

4. Clinical status at enrollment to allow tapering of steroids to equal or less than 0.5 mg/kg/day prednisone (or equivalent).

5. HgB>8.0

6. Pulse oximetry of > 90% on room air

7. Available multivirus-specific VSTs

8. Negative pregnancy test in female patients if applicable (childbearing potential who have received a reduced intensity conditioning regimen).

9. Written informed consent and/or signed assent line from patient, parent or guardian.

2.3.2 Exclusion Criteria

1. Patients receiving ATG, 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 are less than 28 days removed from their allogeneic hematopoieteic stem cell transplant or who have received donor lymphocyte infusions (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. Consent process will consists of two parts: a) screening consent and b) treatment consent

1. Screening consent: The screening portion consists of registering demographic data and patient eligibility data. The search for a suitable matched VST line is initiated if the patient is eligible. If a VST line is not available, the following data will be collected: demographic data, HLA type, infection type and outcome data.
2. Treatment Consent: If a suitable matched VST line is available, the principal investigator or designee discusses the available line with the treating physician. After enrollment in the treatment portion of the protocol the patient can then receive the identified VST line if eligibility criteria are still met.

2.3.4 Donor Eligibility

2.3.4.1 The VST lines that will be used in this third party study derive from two sources

A) Lines that were previously manufactured for our IRB approved protocol H29966. These donors were initially chosen as transplant donors because they were the best match with the original transplant recipient and they met eligibility criteria. There are either additional vials available after the product was used in the donor-specific setting, or was not infused either because the original recipient did not need the VSTs or the original recipient was ineligible. These donors gave consent for lines not used for the original recipient to be used in the third party setting.

B. Additional lines manufactured for third party use from individuals with common HLA types on an IRB approved protocol. 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 antivirus 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 may 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. Those donors will be procured on a separate procurement protocol.

For all third party lines, donors have been evaluated by the 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 have been 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 were deemed to be eligible provided blood for VST generation. For lines that were previously manufactured for H29966, only those where the donor is eligible may be included in the third party bank.

The results of the physician assessment and ID testing were reviewed by a CAGT laboratory director who gave the final eligibility determination according to the SOP for Donor Evaluation.

The processes discussed in the protocol and related manufacturing SOPs are in compliance with 21CFR1271.

2.4 Treatment Plan

2.4.1 VST Line selection and availability

2.4.1.1 VST Lines selection

We will use multivirus-specific VSTs, generated initially for use on our donor-specific study. The lines were generated using a rapid protocol whereby clinical grade pepmixes spanning immunogenic antigens from each of our target viruses are used to directly stimulate PBMCs, followed by expansion in the presence of growth promoting cytokines and the G-Rex culture device optimized for T cell expansion. Donor-specific VST lines generated according to this protocol were previously found to be safe and effective in a Phase I clinical trial as discussed.

To initiate the multivirus-specific VSTs $15x10^6$ PBMCs were pulsed with a master mix of pepmixes spanning Adv (Hexon and Penton), CMV (IE1 and pp65), EBV (LMP2, EBNA1, BZLF1), BK virus

(VP1 and large T) and HHV6 (U90, U11 and U14). After 30mins the cells/pepmix combination was transferred directly to the G-Rex culture device. The VST media was supplemented with the cytokines IL7 and IL4 (10ng/ml and 400U/ml, respectively) in order to inhibit apoptosis and promote expansion of multivirus-specific T cells.

The VST lines were checked for identity, phenotype and sterility, and cryopreserved prior to administration according to our SOP. To test the functional antigen specificity of the VST we will use individual pepmixes spanning each of the viral antigens used in the initial stimulation as a stimulus in IFNγ ELIspot. Epitope mapping will be performed using known HLA epitope peptides or peptide mini-pools to identify novel immunogenic epitope peptides within our target antigens.

Products that meet study specific release criteria, as detailed on the CofA, will be infused as per Section 2.4.2.

If a positive sterility testing result is reported after the product is infused, the FDA and other relevant parties will be notified as per our manufacturing SOP B01.03.XX (Product Quality Assurance Program and Release and Return of Clinical GMP/GTP Products) and clinical research SOP J02.06.XX (Serious Adverse Experience and Unanticipated Problem Reporting). Management of such a situation is further described in our SOP F05.09.XX (Management of Culture Positive Cell Therapy Products).

2.4.1.2 No Matched VST Line Available

If no matched line is available the patient will be registered so that the feasibility of the approach can be assessed.

2.4.1.3 VST Line Available but Patient Status Changes

Patients with a clinical course that changes between screening and infusion and renders patient ineligible at time of infusion will not be given the VST.

2.4.1.4 Criteria for Selection of VST Line

In general preference will be assigned to the infusion of a line with confirmed virus-specific activity against the infecting virus through a shared HLA allele rather than the overall level of HLA match. For example for a patient with an 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. Patients with a partial response are eligible to receive up to 4 additional doses.

2.4.2 Administration and Monitoring

2.4.2.1 Partially HLA-matched VSTs will be thawed and given by intravenous injection. Patients will receive 2 x 10^7partially HLA-matched VSTs/m^2 as a single infusion. In the rare case where insufficient banked cell product is available, a lower number of cells may be infused after discussion with the principal investigator, patient and/or guardian and the treatment team.

2.4.2.2 Premedications: Patients without prior history of reaction to blood products generally do not require premedication. If patients receive premedication, Benadryl 0.25-0.5mg/kg (max 25 mg) IV/po and/or Tylenol 5-10 mg/kg (max 650 mg) po/iv may be given.

2.4.2.3 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

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

2.4.2.5 If a patient 1) receives medication (such as steroids), which may affect the persistence or function of the infused VST, or 2) does not have a complete response to their initial infusion, did not experience a significant toxicity to their initial infusion, and if alternative treatment options are not available or associated with unacceptably high risk of side effects (e.g. count suppression or renal disease), they are eligible to receive up to 4 additional doses at the same initial dose at day 28 post-infusion and at a minimum 2 week interval thereafter. If the additional doses are post steroid administration, the steroid dose must be decreased to a dose of 0.5 mg/kg/day of Prednisone (or equivalent) or lower before the patient may receive additional doses.

Additional doses may be from the same donor or a different donor based on available cell lines and patient/disease factors. Decision to switch to a different donor can be made by the principal investigator based on factors that include sequential treatment of different viral infections, concerns for immune escape of the targeted virus and/or availability of a better matched or otherwise superior VST line. Additional treatments will only be given following the agreement of the patient, treating physician, and investigator. This process can be repeated as needed.

2.5 Risks and Toxicities

2.5.1 Graft Versus Host Disease (GVHD)

The risk that adoptively transferred partially HLA-matched VSTs will cause Grade II or higher GVHD is low according to data from previous studies with 3rd party VSTs specific for adenovirus, EBV and CMV (Section 1.10). If any subject develops GVHD they will receive our standard GVHD treatment as per SOP F08.03.

2.5.2 GVHD Scoring

GVHD will be monitored for 6 weeks following infusion. 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.

Clinical Acute GVHD Assessment

Date _____ Patient ID _____ Karnofsky/Lansky _____

	CODES						DIFFERENTIAL DIAGNOSIS						
	0	1	2	3	4	5		GVHD	Drug Rxn	Cond Reg	TPN	Infect	VOD	Other
Skin	☐	☐	☐	☐	☐		% body rash:____	☐	☐	☐		☐		_____
Lower GI	☐	☐	☐	☐	☐	☐	Vol: _____	☐	☐	☐	☐	☐		_____
Upper GI	☐	☐						☐	☐	☐	☐	☐		_____
Liver	☐	☐	☐	☐	☐		Max bili: _____	☐	☐	☐	☐	☐	☐	_____

Treatment: ☐ CSA ☐ Tacrolimus ☐ Pred ☐ Methylpred ☐ Ontak
☐ Pentostatin ☐ MMF ☐ Etanercept ☐ Other _____

Code Definitions:

Skin:
0 No rash
1 Maculopapular rash, < 25% of body surface
2 Maculopapular rash, 25-50% of body surface
3 Generalized erythroderma
4 Generalized erythroderma with bullous formation and desquamation

Lower GI (Diarrhea):
0 None
1 ≤ 500 mL/day or < 280 mL/m^2
2 501-1000 mL/day or 280- 555 mL/m^2
3 1001-1500 mL/day or 556- 833 mL/m^2
4 > 1500 mL/day or > 833 mL/m^2
5 Severe abdominal pain with or without ileus, or stool with frank blood or melena

Upper GI:
0 No protracted nausea and vomiting
1 Persistent nausea, vomiting or anorexia

Liver (Bilirubin):
0 < 2.0 mg/dl
1 2.1-3.0 mg/dl
2 3.1-6.0 mg/dl
3 6.1-15.0 mg/dl
4 > 15.1 mg/dl

Signature _____

2.5.3 *Chronic GVHD*

Chronic GVHD will be assessed at 3, 6 and 12 months post-infusion. Assessment and a description of symptoms (only if GVHD is present) will be documented through completion of the GVHD symptom record (see Appendix I).

Definite and Possible Manifestations of Chronic GVHD

Organ System	Definite manifestations of chronic GVHD	Possible manifestations of chronic GVHD
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

2.5.4 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 4.X with the exception of CRS that is related to T-cell infusions. CRS will be graded according to Appendix II.

2.5.5 Management of Toxicity

VSTs are susceptible to killing by steroids given 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 VST administration. Anti TNF-alpha receptor antibody and anti IL-6 also may have some efficacy in individuals that develop SIRS after T cell infusion and may be considered in patients with consistent clinical signs/symptoms (see SOP: F05.11.4 MANAGEMENT OF

ADVERSE REACTIONS TO CTL INFUSIONS for dosage details). Other supportive care would be per standard medical practice.

3 STUDY ENDPOINTS

3.1 Primary Endpoint

The primary objectives for this pilot study are feasibility and safety. Safety of administration of VSTs is 42 days for GVHD and 28 days for other toxicities. Except as noted below, the safety endpoint will be defined as acute GvHD grades III-IV within 42 days of the last dose of VSTs or grades 3-5 non-hematologic adverse events related to the T cell product within 28 days of the last VST 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 4.X.

Grade 3 and 4 expected reactions seen with the use of T cell-based immunotherapy, such as fever and hypotension requiring pressor support, will not be considered a safety endpoint. Any other grade 3 or greater toxicity felt to be related to or resulting from Cytokine Release Syndrome (CRS) is included in the safety endpoint. Grade 3 and 4 CRS infusion reactions that are persistent beyond 72 hours will be reported to the FDA in an expedited fashion, and will be considered a treatment-limiting toxicity.

Toxicities to consider include GI toxicity, renal toxicity, hemorrhagic toxicity, cardiovascular toxicity (hypotension, cardiac arrhythmia and left ventricular systolic dysfunction), neurological 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).[59]

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

3.1.2 Antiviral Activity

Peripheral blood and, where relevant, stool and urine will be monitored for CMV, EBV, adenovirus, BK virus, JC virus and/or HHV6 viral load. 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 and clinical signs and symptoms.
Partial response: Decrease in viral load of at least 50% from baseline or 50% improvement of clinical signs and symptoms
Mixed response: Decrease in viral load of at least 50% from baseline for one infection and an increase or no change in viral load for a second infection (only applicable for patients with two or more infections at baseline).
Stable disease: Changes insufficient to qualify as partial response or progression
Progression: Increase in viral load of at least 50% from baseline or dissemination to other sites of disease.

Viral response data will be reviewed by a consensus committee of investigators.

3.1.3 Reconstitution of Antiviral Immunity

Patient serum and peripheral blood mononuclear cells will be monitored for virus-specific activity by functional studies including ELIspot with appropriate viral specific peptide mixtures and available HLA-restricted epitope peptides, intracellular cytokine staining, serum cytokine profiling and/or other assays as they become available for immune profiling purposes.

3.1.4 Persistence of infused VSTs

Persistence of infused T cells will be monitored using deep sequencing and additional tests as indicated to track the TCR v-beta repertoire in the patient peripheral blood prior to and post-infusion.

3.1.5 *Effects on Clinical Signs of Viral Infection*

If a patient has organ involvement clinical response will be monitored. For patients with EBV lymphoma and measurable disease, response will be assessed by RECIST criteria.

3.1.6 Survival

Overall survival at 3, 6 and 12 months post VST infusion will be computed.

3.1.7 Chronic GVHD

Chronic GVHD will be assessed 3, 6 and 12 months post VST infusion.

3.1.8 Viral Reactivations

All CMV, EBV, adenovirus, BK virus, JC virus and HHV6 infections/reactivations occurring within 12 months of VST infusion will be collected. Information about infection or reactivation with other viruses may be collected as well.

3.1.9 Secondary graft failure

Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in the ANC to <500/mm^3 for three consecutive measurements on different days, unresponsive to growth factor therapy that persists for at least 14 days in the absence of a known cause such as relapse. Secondary graft failure will be assessed at 30 days post VST infusion.

4 PATIENT ENROLLMENT AND EVALUATION

4.1 Enrollment

The informed consent process will begin at recognition of subject eligibility and consent will be obtained per institutional practices before study therapy is initiated. This protocol will be discussed with eligible patients and, when appropriate, their guardians. Only patients who receive their care in the integrated Cell and Gene Therapy Transplant program at Texas Children's Hospital and Methodist Hospital are eligible for this study. Consent process will consists of two parts: a) screening consent and b) treatment consent

1. The screening portion consists of registering demographic data and patient eligibility data. The search for a suitable matched virus VST line is initiated if the patient is eligible. If a VST line is not available, the following data will be collected: demographic data, HLA type, infection type and follow up data.
2. If a suitable matched VST line is available, the principal investigator or designee discusses the available line with the treating physician. After enrollment in the treatment portion of the protocol patient can then receive the identified VST line if eligibility criteria are still met.
3. Patients will be assessed for eligibility for subsequent infusions by completing the eligibility form. If the patient is deemed eligible, the search for a subsequent VST line will be initiated.

All cell culture manipulations will be carried out in the Center for Cell and Gene Therapy GMP facility using current standard operating procedures (SOPs). After Quality Assurance testing is complete a Certificate of Analysis will be issued.

4.2 Study Monitoring

4.2.1 Follow-up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 4.2.2

Follow-up Assessments: The timing of follow-up visits is based on the date of VST infusion. If a patient has multiple VST doses the schedule resets again at the beginning so follow up relates to the last VST dose. Additional follow up assessments may be done based on clinical and laboratory responses.

Table 4.2.1
FOLLOW-UP SCHEDULE

Assessment Time	(Days Post-Infusion)
1 week	7 days
2 weeks	14 days
3 weeks	21 days
4 weeks	28 days
5 weeks	35 days
6 weeks	42 days
3 months	90 days
6 months	180 days
12 months	365 days

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, HHV6, JC virus and BK virus
3. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests
4. CBC with differential, platelet count
5. Basic chemistry
6. Liver function tests (bilirubin, alkaline phosphatase, AST, ALT)
7. Tacrolimus/cyclosporine level if on these agents
8. Pregnancy test* if female patient of childbearing potential and has received a reduced intensity transplant regimen
9. Samples for research laboratory studies*

Post-Infusion

1. Viral load for the treated virus or viruses will be collected weekly at 1, 2, 3, 4 and 6 weeks, and 3 months post-infusion.Thereafter, viral load results will be followed until 12 months post infusion as clinically indicated. Additional time points may be analyzed as clinically indicated. If the treatment team is also measuring viral loads for other viruses we will follow these results.
2. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests weekly until Day 42

3. Chronic GVHD evaluation (if present) at 3, 6, and 12 months
4. Liver function tests (bilirubin, alkaline phosphatase, AST, ALT) at 1, 2, 3, 4, 5 and 6 and 3 months.
5. Infusion-related toxicities within 24 hours and toxicity evaluation weekly until Day 30, and acute GVHD until Day 42.
6. Steroid dose weekly until Day 42, and at 3 and 6 months
7. Samples for laboratory studies at 1, 2, 3, 4, and, 6 weeks and 3 months: 30-40mls or 6-8 teaspoons will be collected at each time point. For recipients who weigh less than 10kg the amount will be reduced to 3mls/kg. Depending on clinical and laboratory viral responses samples may be collected at additional time points.*

Research laboratory studies will include:
Assessment of virus-specific immunity as measured by serum profiling, ELIspot, intracellular cytokine staining, multimer assays and/or other assays as they become available.

After the initial follow up for safety is complete at 30 days for toxicity and 42 days for GVHD, patients will continue to be followed per routine clinical care post transplant. We will perform laboratory follow-up studies for 3 months post-infusion or longer depending on clinical and laboratory viral responses and will assess these patients clinically at 3, 6 and 12 months to evaluate for any long term effects attributable to the VST infusion.

Table 4.2.2 REQUIRED ASSESSMENTS

	Baseline (Day 0)	7	14	21	28	35	42	90	180	365
History and physical exam	X									
CMV, EBV, adenovirus, HHV6 and/or BK virus load (if previously positive)	X	X	X	X	X		X	X		
Acute GVHD evaluation	X	X	X	X	X	X	X			
Chronic GVHD evaluation								X	X	X
Liver function tests (alkaline phosphatase, bilirubin, AST, ALT)	X	X	X	X	X	X	X	X		
Tacrolimus/cyclosporine levels*	X									
Pregnancy test[1]	X									
Infusion related toxicity evaluation	X[2]	X	X	X	X[3]					
Steroid dose	X	X	X	X	X	X	X	X	X	
Blood and serum for ancillary laboratory studies	X[4]	X[4]	X[4]	X[4]	X[4]		X[4]	X[4]		
Infections	X	X	X	X	X		X	X		
CBC with Differential	X									
Basic Chemistry	X									

***** If on these agents

[1] Pregnancy test if of child bearing potential and has received a reduced intensity transplant

[2] This evaluation should be done within 24 hours of infusion

[3] This evaluation should be done on day 30.

[4] Research procedures beyond that required for usual care - 30-40mls or 6-8 teaspoons will be collected at each time point. For recipients who weigh less than 10kg the amount will be reduced to 3mls/kg. Depending on clinical and laboratory responses samples may be collected at additional time points before or after 90 days.

4.3 Modified Follow-Up & Off Study Criteria

4.3.1 Criteria for Modified Follow-Up

The following criteria will result in the patient being ineligible for further treatment on the protocol, although response data will continue to be collected as applicable:

4.3.1.1 Any patient who develops irreversible, life threatening, Grade 3-4 acute GvHD within 42 days, Grades 3-5 non-hematologic adverse event within 28 days from last VST dose and considered to be primarily related to VST injection. In such patients, the toxicities will be followed until resolution or until their off study date.

4.3.1.2 Any patient who receives any other hematopoietic cell product. In such patients, adverse event data collection will cease.

4.3.1.3 Any patient who receives therapy for relapse of their primary malignancy.In such patients adverse event data collection will cease.

4.3.1.4 Any patient who experiences Grade 3 or 4 cytokine release syndrome that persists beyond 72 hours. In such patients, the toxicity will be followed until resolution or until their off study date.

Patients who meet “modified follow-up” criteria remain on long-term follow-up as per the required assesments table 4.2.2.

4.3.2 Off Study Criteria

4.3.2.1 Completion of study specified procedures.
4.3.2.2 Refusal of further study follow-up by patient or legal guardian
4.3.2.3 Lost to follow up
4.3.2.4 Death

5 STATISTICAL CONSIDERATIONS

5.1 Study Design Synopsis

This study is designed as a single-arm pilot study to evaluate the feasibility, safety and efficacy of most closely HLA matched multivirus specific VST lines (CHM-VSTs) in HSCT patients with either of five viruses; BK virus, HHV6, EBV, CMV and adenovirus persistent despite of standard therapy. The study first conducts a phase IIA trial to evaluate the clinical response rate. After the phase IIa trial, if the data suggests excellent clinical responses that warrant further investigation, the study will be expanded to recruit 30 additional patients to evaluate the role of HLA matching on viral outcomes.

The total sample size of the study will be a maximum of 80 patients who receive at least one VST infusion. The sample size for the phase II A trial is 50 patients who receive at least one VST infusion. A larger number of potential subjects will be screened to assess the feasibility of the approach.

We expect to enroll 15-20 patients per year and therefore expect the study to conclude within the estimated accrual period of 4 years.

5.2 Sample Size Determination and Design Characteristics

Frist, the study will conduct a single arm poilt study using a phase IIa design. Simon's two-stage Minimax design[60] will be used to for the single-arm pilot study. Under the null hypothesis that the VST is not active, the data from previous trials suggest that the true response rate is 20% in anti-viral activity, where a positive response is defined as the viral load reduction to the normal level for at least one of the 5 virus types (CMV, EBV, adenovirus, BK virus and HHV6) within 42 days following the initial treatment of VST.

Allowing a 5% type-I error and 90% power, the null hypothesis will be tested against a one-sided alternative that the true response rate is 40%. The maximum size is 45 eligible patients, defined as the patients who receive the VST infusion and who can finish the 42 days follow-up. We anticipate that most patients will finish the follow up and will allow a 10% drop out rate. Therefore, the maximum sample size is 50.

The phase IIa trial operates in two stages, where the first stage recruits 24 patients. The trial pauses at the end of the first stage to make the decision on whether to stop the trial early due to lack of enough efficacy for treating viral infection, according to the rule as follows. Of the 24 patients at the first stage, if 5 or fewer positive responses are observed, then the study will be stopped. Otherwise, 21 additional patients will be accrued to reach the goal of 45 accruals. At the end of the trial, the null hypothesis will be rejected, if 14 or more positive responses are observed in among the 45 patients. This design has 65.6% chance to stop early when the true response rate is 20%.

After the phase IIa trial suggests excellent clinical responses that warrant further investigation, the study will be expanded to recruit 30 additional patients to evaluate the viral reduction based HLA matching. We hypothesize the viral reduction among the low HLA matching group (1/8, 2/8, or 3/8 HLA matching) is not worse than the high HLA matching group (4/8, 5/8, 6/8, 7/8, or 8/8).

The sample size is calculated based on non-inferiority trial for comparing two-sample means. Based on the preliminary data, the standard deviation of viral reduction is about 18. We assume that the viral reduction in the low HLA matching group is not inferior to the high HLA matching group when the difference in the viral reduction between the two groups is no less than -10 (non-inferiority margin). Preliminary data suggest that the ratio of sample size between the low and high HLA matching groups is 5 to 7. A sample size of 30 patients with low HLA matching and 42 with high HLA match (total 72) would have at least 85% power to test the non-inferioirty hypothesis with 2-side 5% alpha level. Assuming 10% of patients to be lost to follow-up, then the study will recruit up to a total of 80 patients.

5.3 Stopping rule and data monitoring

The phase IIa portion of the trial will be stopped for the lack of efficacy with one interim analysis to be conducted when the first 24 eligible patients have finished their 42 days follow up. The stopping rule is specified in Section 5.2.

The phase IIa portion of the trial will monitor the dose-limiting toxicities (DLT), defined as acute GvHD grades III-IV within 42 days following the administration of VSTs or grades 3-5 non-hematologic adverse events that are at least possibly related to the T cell product within 28 days of the last dose 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 4.X.
*Note - Grade 3 and 4 expected reactions seen with the use of T cell-based immunotherapy, such as fever and hypotension requiring pressor support, will not be considered a DLT. Any other grade 3 or greater toxicity felt to be related to or resulting from Cytokine Release Syndrome (CRS) is included in the DLT. Grade 3 and 4 CRS infusion reactions that are persistent beyond 72 hours will be reported to the FDA in an expedited fashion, and will be considered a treatment-limiting toxicity.

The proportion of events will be monitored using a stopping guideline based on a Pocock-type boundary for repeated testing for toxicity. [61] The risk of DLT is considered to be moderate in this population, assumed to be 25%. The following sequential boundaries will be used to monitor dose-limiting toxicity rate:

# pts	1-3	4	5-7	8-9	10-11	12-14	15-17	18-20
DLT<	--	4	5	6	7	8	9	10
# pts	21-22	23-25	26-28	29-31	32-34	35-37	38-40	41-43
DLT<	11	12	13	14	15	16	17	18
# pts	44-46	47-49	50					
DLT<	19	20	21					

The trial will be stopped if the cumulative number of patients with DLT is equal to or exceeds the boundary listed in the above table. This stopping rule yields the probability of crossing the boundary at most 5% when the true DLT rate is 25%, where the expected number of DLT is about 12. The probability of crossing the boundary is 62.7% is when the true DLT rate is 40%.

If a patient experiences Grade 5 toxicity that is at least possibly related to the product, we will hold all infusions on the study until both the incident in question and the infused product have undergone a thorough internal review and the outcome discussed with the FDA.

5.4 Data Analysis

Safety and toxicity outcomes including adverse events, GvHD, clinical signs of viral infections, secondary graft failure and laboratory measurements will be summarized using descriptive statistics (frequency table, means, standard deviations, medians and ranges). Toxicity information including the type, severity, time of onset, time of resolution, and the probable association with the study regimen will be tabulated and summarized.

Response rate including complete response, partial response and mixed response will be summarized as frequency table. The response rate with 95% CI will be reported. Reconstitution of antiviral immunity using multimer analysis and ELIspot assays will also be summarized using descriptive statistics at each time point. Pairwise comparisons will be performed to compare changes of these immunological parameters from VST infusion to each time point of post-infusion measurements using paired t-tests or Wilcoxon signed-ranks tests. The normality assumption will

be assessed and transformations to achieve approximate normality will be carried out if necessary. Overall survival post VST infusion will be analyzed by the Kaplan-Meier method.

Viral load reduction will be compared between the low and high HLA using the two-sample t-test. Additionally, Viral load levels will be correlated with immunological parameters using correlation coefficients. Longitudinal analysis is employed to model repeatedly-measured immunologic parameters. This will allow us to model patterns of immune response per patient while allowing for varying intercepts and slopes for a patient. Viral load levels will be correlated with immunological parameters using correlation coefficients. These modeling strategies will be considered exploratory in nature due to the limited patient numbers in this initial trial.

Overall survival since allogeneic hematopoietic stem cell transplant will be analyzed using the Kaplan-Meier method. Cumulative incidences to the time of complete/partial resolution of viral infection and viral load reduction, considering death a competing event, will be analyzed in univariate and multivariate model using the Fine and Gray method.

6 REPORTING REQUIREMENTS

6.1 Registration

Register all patients with Cell and Gene Therapy Research Coordinator.

The following forms should be completed:

- Eligibility check list
- On study form
- Concomitant medication
- Off study form
- Response Form
- Adverse event form
- CRS Adverse Event Form (as applicable)
- Death form

6.2 Drug Toxicity and/or Adverse Reactions

6.2.1 Adverse events will be collected as per SOP J 02.05.XX and J02.75.XX. Data on adverse experiences/toxicities regardless of seriousness must be collected for documentation purposes only for 28 days after the last dosing of the study drug/biologic with the exception of acute GVHD which will be followed for 6 weeks.

6.2.2 Serious adverse events will be collected and reported as per SOP J 02.06.XX until 1 year after the last dosing of study drug/biologic.

6.3 Informed Consent

All patents and/or their legal guardian must sign a document of informed consent consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted

for treatment without such a document signed by him or his legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines

6.4 Clinical Trial Oversight & Monitoring

This protocol will be conducted in accordance with the Cell Therapy Monitoring Plan on file with the FDA.

This protocol will be monitored in accordance with the current Data Safety Monitoring Plan for investigator-initiated Phase I and II studies in the Center for Cell and Gene Therapy at Baylor College of Medicine.

The conduct of this clinical trial will be evaluated in accordance with the Texas Children's Cancer Center and Center for Cell and Gene Therapy Quality Assurance Policy and Procedure Plan.

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Appendix I

CHRONIC GVHD ASSESSMENT

Patient's Name:_____

Date of Chronic GVHD Evaluation: _____

Time point of Evaluation (post cell infusion): ☐ Day 90 ☐ Day 180 ☐ Day 365

If the patient does not have symptoms of Chronic GVHD (cGVHD), please mark "None" below and sign and date this page. The assessment table on the attached page does not need to be completed if the patient does not have cGVHD. If the patient does have symptoms of cGVHD, please record the grade and severity below, sign and date this page and complete the assessment table on the attached page.

Maximum Grade of Chronic GvHD: ☐**NONE**
(since the last assessment)
☐**LIMITED** localized skin involvement and/or hepatic dysfunction due to cGVHD
☐**EXTENSIVE** one or more of the following:
--generalized skin involvement; or
--liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or
--involvement of eye: Schirmer's test with < 5 mm wetting; or
--involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or
--involvement of any other target organ

Overall Severity of Chronic GVHD: ☐**NONE**
(since the last assessment)
☐ **MILD** signs /symptoms do not interfere substantially with function and do not progress once appropriately treated with local therapy or standard systemic therapy (corticosteroids and/or cyclosporine or FK506)
☐**MODERATE** signs/symptoms interfere somewhat with function despite appropriate therapy or are progressive through first line systemic therapy (corticosteroids and/or cyclosporine or FK506)
☐**SEVERE** signs/symptoms limit function substantially despite appropriate therapy or are progressive through second line therapy

The following section is to be completed ONLY at the time of the INITIAL ONSET of Chronic GVHD:

Date of Initial Onset of Chronic GVHD: _____
☐Progressive (aGVHD progressed directly to cGVHD)
☐Interrupted (aGVHD resolved, then cGVHD developed)
☐De novo (aGVHD never developed)
☐Chronic GVHD flare (symptoms reactivated within 30 days of drug tapering or discontinuation)

The following section is to completed IF patient has aGVHD symptoms post Day 100:
Did Acute Graft vs. Host Disease (aGVHD) develop or persist (or a flare-up that was more severe) since the date of the last assessment? ☐**YES** ☐**NO**

Additional Information/Comments: _____

_____

_____ _____
Printed Physician Name Physician Signature/Date

Patient's Name:_____

Stage	0	1	2	3
1. Skin	☐ No skin changes (a-f)			
a. Rash	☐ No changes	☐ < 10% of BSA	☐ 10 - 50% of BSA	☐ > 50% of BSA
b. Lichenoid changes	☐ No changes	☐ < 10% of BSA	☐ 10 - 50% of BSA	☐ > 50% of BSA
c. Sclerodermatous changes	☐ No changes	☐ < 10% of BSA	☐ 10 - 50% of BSA	☐ > 50% of BSA
d. Hyper or hypopigmentation	☐ No changes	☐ < 10% of BSA	☐ 10 - 50% of BSA	☐ > 50% of BSA
e. Dry, flaky skin	☐ No changes	☐ < 10% of BSA	☐ 10 - 50% of BSA	☐ > 50% of BSA
f. Alopecia	☐ None	☐ Mild alopecia	☐ Pronounced alopecia	
2. Joints	☐ No joint problems (a,b)			
a. Contractures	☐ No contractures	--	☐ Mild joint contractures (do not affect ADL)	☐ Severe joint contractures (affect activities of daily living)
b. Arthralgias/arthritis	☐ None	☐ Pain and/or inflammation not interfering with function	☐ Pain and/or inflammation with either pain or pain meds interfering with function	☐ Pain and/or inflammation with either pain or pain meds interfering with activities of daily living
3. Oral	☐ No changes	☐ Painless erythema and/or lichenoid changes	☐ Diffuse erythema, ulcers, and/or lichenoid changes with pain; able to eat most foods	☐ Diffuse erythema, ulcers, and/or lichenoid changes with pain; unable to eat most foods
4. Ocular	☐ No changes	☐ Dryness of eyes, mild, not requiring treatment and/or asymptomatic keratoconjunctivitis	☐ Dryness of eyes requiring artificial tears or lacrimal plugging and/or symptomatic keratoconjunctivitis (ie. pain, irritation)	☐ Keratoconjunctivitis with pseudomembranes, corneal ulcerations, or loss of vision
5. Hepatic				
a. ALT (SGPT)	☐ <= 2.5 x ULN	☐ > 2.5 – 5 x ULN	☐ > 5 – 20 x ULN	☐ > 20 x ULN
b. Bilirubin	☐ <= 1.5 x ULN	☐ > 1.5 – 3 x ULN	☐ > 3 – 10 x ULN	☐ > 10 x ULN
c. Alkaline phosphatase	☐ <= 2.5 x ULN	☐ > 2.5 – 5 x ULN	☐ > 5 – 20 x ULN	☐ > 20 x ULN
6. Esophagus	☐ No dysphagia or odynophagia	☐ Mild dysphagia, but can eat regular diet	☐ Dysphagia or odynophagia (painful swallowing) requiring dietary changes	☐ Dysphagia requiring parenteral nutrition and/or web/stricture formation
7. Gastrointestinal	☐ No GI symptoms (a-c)			
a. Vomiting (chronic)	☐ No chronic vomiting	☐ Vomiting, average 1x/24 hrs x > 1week	☐ Vomiting, average 2-5 x/24 hrs x > 1week	☐ Vomiting, average ≥ 6x/24 hrs x > 1week
b. Diarrhea (chronic)	☐ No chronic diarrhea	☐ Increase of 2 -3 stools/day more than normal x > 1week	☐ Increase of 4-6 stools/day more than normal, or nocturnal stools x > 1week	☐ Increase of ≥ 7 stools/day more than normal, or incontinence, or need for parenteral support for dehydration x > 1week
c. Abdominal pain (chronic)	☐ No chronic abdominal pain	☐ Mild pain/cramps, not interfering with function	☐ Moderate pain/cramps; pain or analgesics interfering with function, but not ADL	☐ Severe pain; pain or analgesics interfering with function and ADL
8. Weight loss	☐ < 5% weight loss	☐ 5-10% loss	☐ 10 - <20% loss	☐ ≥ 20% loss
9. Pulmonary	☐ No pulmonary changes (a-e)			
a. FVC	☐ > 75% of predicted	☐ ≥ 50 - 75% of predicted	☐ ≥ 25 - <50% of predicted	☐ < 25% of predicted
b. FEV_1 (+FEV1/FVC<0.8)	☐ > 75% of predicted	☐ ≥ 50 - 75% of predicted	☐ ≥ 25 - <50% of predicted	☐ < 25% of predicted
c. $DLCO_2$ (corrected)	☐ > 75% of predicted	☐ ≥ 50 - 75% of predicted	☐ ≥ 25 - <50% of predicted	☐ < 25% of predicted
d. Hypoxia	☐ Normal	☐ ↓ O2 sat with exercise	☐ ↓ O2 sat at rest, needs O2	☐ Needs CPAP or vent
e. Dyspnea	☐ No dyspnea	☐ Dyspnea with exertion	☐ Dyspnea with normal activities	☐ Dyspnea at rest or requiring ventilator support
10. Platelets	☐ Platelets ≥ 100,000	--	☐ Thrombocytopenia, platelets 50,000-99,000	☐ Severe thrombocytopenia, platelets < 50,000
11. Musculoskeletal	☐ No serositis or myositis (a,b)			
a. Serositis	☐ None	☐ Asymptomatic	☐ Symptomatic, requiring medical intervention or single aspiration	☐ Symptomatic, requiring surgical intervention, drain, or window
b. Myositis	☐ None	☐ Pain or weakness not interfering with function	☐ Pain or weakness interfering with function but not ADL	☐ Pain or weakness interfering with activities of daily living
12. Performance (Karnofsky/ Lansky score)	☐ 90-100	☐ 70-80	☐ 50-60	☐ <50

Physician Signature: _____ Date: _____

Appendix II CRS Grading Scale

Grade	Symptoms
1	• Symptoms are not life threatening and require symptomatic treatment only (e.g. fever, nausea, fatigue, headache, myalgia, malaise)
2	• Symptoms require and respond to moderate intervention • Oxygen requirement <40% or hypotension responsive to fluids or • low dose of one vasopressor or Grade 2 organ toxicity
3	• Symptoms require and respond to aggressive intervention • Oxygen requirement ≥ 40% or hypotension requiring high dose or multiple vasopressors or • Grade 3 organ toxicity or Grade 4 transaminitis
4	• Life-threatening symptoms • Requirements for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
5	• Death