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Cincinnati Children's Hospital Medical Center
Bone Marrow Transplant and Immune Deficiency

Donor-Derived Viral Specific T-cells (VSTs) for Prophylaxis against Viral Infections after Allogeneic Stem Cell Transplant

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1. ABSTRACT

Viral reactivation and infection is a major cause of morbidity and mortality after stem cell transplant. In this study we will attempt to decrease the incidence of viral infection or reactivation following stem cell transplantation. Peripheral blood from the transplant recipient's donor will be used to generate viral specific T-lymphocytes (VSTs) with specificity for EBV, CMV, BKV, and adenovirus. The VST's will be frozen, and then infused no earlier than 21 days following stem cell transplantation. The primary study endpoint is feasibility and safety of infusion of the cells 21 days post-transplant. The secondary endpoint is efficacy, as measured by incidence of viral infection and/or reactivation and by the persistence of viral specific T-cells in the blood.

2. PURPOSE

2.1 Objective of Study

The objective of this study is to establish the safety of infusing VSTs 21 days following stem cell transplant. A secondary objective is to assess efficacy of the viral cells in preventing viral reactivation or infection.

2.2 Primary Endpoint: The primary endpoint of the study is the ability to give VSTs to stem cell transplant recipients 21 days after stem cell infusion without toxicity or an increased incidence of acute Graft-Vs-Host Disease (aGVHD).

2.3 Secondary Endpoint: The secondary endpoint of the study is clinical efficacy of the infused viral specific T-cells in preventing the development of viral infections. Efficacy will be assessed by comparing the incidence of viral infection in recipients of VST received as prophylaxis compared to a matched historical group of SCT recipients.

2.4 Significance

Reactivation of viruses after stem cell transplant is a major cause of morbidity and mortality. Moreover, drugs used to suppress or control infections have significant toxicities, are expensive, and often prolong the need for hospitalization. The availability of viral specific T-cells has the potential to improve survival, decrease the use of anti-viral medications and reduce morbidity and cost associated with viral infections.

3. PREVIOUS WORK IN THIS AREA

Viral infections are a common problem after hematopoietic stem cell transplant (HSCT), and cause significant morbidity and mortality. Moreover, treatment of viral infections is expensive and time consuming, with families often administering prolonged treatments with intravenous anti-viral medications, or children requiring prolonged admissions to the hospital.

Review of the experience at Cincinnati Children's Hospital has demonstrated a high burden of viral infections.

One hundred and twenty four allogeneic transplant patients, median age 4.9 years (range: 0.2-25.4) were identified and charts retrospectively reviewed. Ninety-five patients underwent HSCT for non-malignant disease, 29 for malignant disease. Graft source was unrelated donor in 102 (82%) and matched related in 22 (18%). Stem cell source was bone marrow in 94 (76%), peripheral blood stem cells in 16 (13%) and cord blood in 14 (11%). Sixty-four patients (35 with non-malignant disease, 29 with malignant disease) received a myeloablative conditioning regimen. Forty-six patients (all with non-malignant disease) received a reduced-intensity conditioning (RIC) regimen, 14 Fanconi anemia patients received a RIC regimen with T-cell depletion of the graft. All patients were monitored for cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus (ADV) weekly in blood by PCR testing until day +100. Human Herpes Virus-6 (HHV-6) and BK virus (BKV) were monitored when clinically indicated. Viral infection was defined as quantitative PCR detection in blood or qualitative detection in stool, urine, body fluids or relevant tissue (Ozdemir 2012).

Table 1 demonstrates the prevalence and features of most common viral infections. Overall survival was 70% with viral infection as the cause of death in 12 patients (10%).

Table 1. Viral infections

Virus	**ADV**	**EBV**	**CMV**	**BKV**	**HHV-6**
Prevalence	66/124 (53%)	47/124 (38%)	25/124 (20%)	26/48 (54%)	23/77 (30%)
Viremia only (n)	16	35	16	1	7
Disseminated disease (n)	29	9	8	14	4
Reduced-intensity conditioning	39/60 (65%)	19/60 (32%)	13/60 (22%)	7/20 (35%)	14/40 (35%)
Myeloablative conditioning with ATG	26/55 (47%)	26/55 (47%)	12/55 (22%)	19/26 (73%)	8/32 (25%)
Myeloablative conditioning without ATG	1/9 (11%)	2/9 (22%)	0 (0%)	0/2 (0%)	1/5 (20%)
Fanconi anemia (T-cell depleted)	5/14 (36%)	4/14 (29%)	4/14 (29%)	6/9 (67%)	2/7 (29%)
Median post-HSCT day of viral detection (range)	26 (0-341)	42 (4-763)	15 (0-78)	36 (13-119)	86 (1-419)

*Viremia was defined as the presence of virus in blood. Disseminated infection was defined as virus detection in at least two different organ systems. N: patients with a specified condition, (%) percentage of patients affected. Myeloablative conditioning regimen: Busulfan/Cyclophosphamide/Anti-thymocyte Globulin (ATG) or Cyclophosphamide/TBI. Reduced-intensity conditioning regimen: Alemtuzumab/Fludarabine/Melphalan

Adenovirus and BK virus were the most prevalent infections, detected in half of the transplant patients, with adenovirus most likely to be disseminated. The use of Alemtuzumab, ATG and T-cell depletion is associated with increased risk of viral infection. We observed a higher prevalence of Adenovirus infections compared to other reports, likely due to high percentage of unrelated donors and use of Alemtuzumab and

ATG in these patients. These data reinforce the importance of adenovirus as a potential pathogen causing a high rate of disseminated infection in transplant recipients. Currently, there are no effective drugs for treatment of disseminated adenovirus infection (Ozdemir 2012).

A more recent analysis was done with one hundred and eighty seven allogeneic transplants performed at this institution between 2015-2018. Excluded from this analysis were the approximately 10% of patients who are at low risk for viral infections due to having fully matched related donors and a conditioning regimen that did not use T cell depletion (either alemtuzumab or ATG). Of these 153 of the 183 patients receiving T cell depletion had at least one positive result for at least one of the four viruses. The overall prevalence for each virus was in line with the prior analysis cited above.

A series of ground-breaking studies led by Drs. Bollard and Heslop at Texas Children's Hospital (TCH) have demonstrated that T-lymphocyte (VST) lines with specificity for commonly detected viruses can be safely administered to pediatric transplantation recipients receiving allogeneic stem cell transplants without inducing graft-versus-host disease. In a pivotal study, the TCH group have shown that trivirus-specific VST lines targeting CMV, EBV, and adenovirus can be produced by genetically modified activated monocytes and EBV transformed B-lymphoblastoid cell lines (EBV-LCL) using a chimeric adenoviral vector expressing the immunodominant CMV-PP65 antigen.

Further developments in the production of tri-valent VSTs have dramatically reduced production time resulting in the generation of VSTs for clinical use. The team at TCH have shown that clinically effective VSTs can be generated in 2-3 weeks by stimulating PBMCs using peptides containing target antigens from the individual viruses, resulting in generation of viral specific VSTs in a shorter time frame compared to previously used methods. Using this technology, the group showed an overall response rate of 80% against viral infections including adenovirus, EBV and CMV with no immediate or delayed toxicity (Gerdemann et al, 2013).

A phase I/II trial investigating the safety and efficacy of VSTs derived from patients' stem cell donor has been open at CCHMC since 2014. Since then, more than 100 patients have enrolled and more than 80 infusions have been given to more than 50 patients for the treatment of viral infection. In this protocol, VSTs can be given no earlier than 28 days after stem cell infusion. **There has been no infusional toxicity or associated GVHD seen in any of these patients at this time point.** Since June 2017, all VSTs at CCHMC have been manufactured via the method of stimulation of PBMCs with viral peptides as discussed above. This has rapidly increased the turnaround time for generation of cell lines as the manufacturing process consistently takes only 2-3 weeks. Looking retrospectively at the cohort of patients who have received donor derived VSTs at our institution since the manufacturing process was changed to the current method, the median time to viral positivity following stem cell transplant was 22 days for CMV, 35 days for EBV, 49 days for adenovirus, and 21 days for BK virus. On our currently open trial using donor derived VSTs, cells may be infused no earlier than 28 days after transplantation. Recently, we have analyzed the kinetics of viral

reactivation in the first 22 patients who received VSTs manufactured using the current rapidly generated, pep-mix based method. Analysis revealed that a substantive amount of viral disease first manifests between the 21st and 28th day following transplant. As a result, 10/22 patients would have been eligible to receive prophylactic VSTs at day +21, whereas only 5/28 would have been eligible at day +28.

Currently, there is no published literature on the use of VST infusions to prevent or decrease the incidence and/or severity of viral infection or reactivation following stem cell transplant. However, a recent abstract was presented showing in a phase I clinical trial, the infusion of VSTs early after transplant (range of day +2-day +52) was feasible and safe, with no dose limiting toxicities and the only cases of GVHD occurring in patients with elevated GVHD biomarkers prior to the infusion of cells (Muranski et al 2018). If successful, this approach would be expected to decrease morbidity along with health care cost to the patient with less need for antiviral medications (and their associated toxicities) and potentially shortened hospital stays.

4. MANUFACTURE OF THE VSTs

The investigational cell product is an EBV, Adenovirus, CMV, and BKV-specific cytotoxic T lymphocyte preparation (quadravalent VSTs) derived from the recipient's stem cell transplant donor. At the time of infusion, the product is comprised of:

Donor-derived Cytotoxic T Lymphocytes (VST)

Human Serum Albumin

Plasmalyte

CryoStor 5 (Contains DMSO)

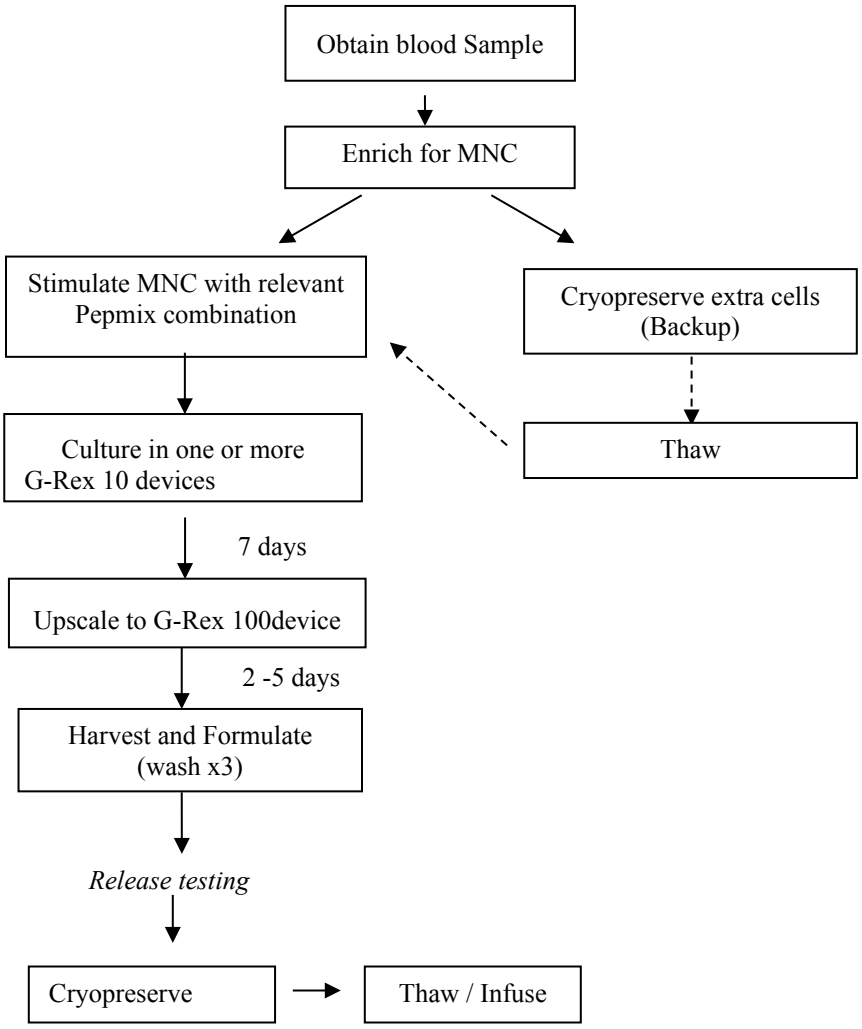
Dextran

The concentration and total cell number of VST in the product will vary based on the recipient body weight and the dose requested.

All processing operations are performed by the Cell Therapy Division at Hoxworth Blood Center. This department routinely performs a variety of cell processing services such as cryopreservation and targeted cell selections to support clinical trial and standard of care activities for the stem cell transplant programs at UC Health, Cincinnati Children's Hospital Medical Center (CCHMC), Jewish Hospital, Inc., in Kenwood, OH, and Akron Children's Hospital in Akron, Ohio. Hoxworth Blood Center's Cellular Therapies processing laboratory has been FACT- accredited since 2003 and AABB accredited since 2005.

A flow diagram of the manufacturing process is provided in Figure 8.1.

Figure 8.1 Processing flow chart for VST generation.



Donor selection and consent activities are performed by the clinical staff at CCHMC or the NMDP under a separate CCHMC IRB protocol (CCHMC IRB# 2013-2777). All donors will be screened and tested for a panel of infectious diseases within 7 days of collection (HIV I & II ag, HTLV I & II, RPR, Hepatitis B, Hepatitis C, and CMV) using an FDA-approved testing method. Up to eighty mls of peripheral blood will be collected from donors and transported directly to Hoxworth Blood Center for production of the investigational product. Briefly, the mononuclear cells are isolated and exposed to a mixture of antigenic viral peptides (pepmixes) that stimulate reactive T lymphocytes to proliferate in culture over a 10 -12 day period, at which point the VSTs are harvested, cryopreserved, and stored in LN_2 until needed. When requested, provided all release testing criteria have been met, the product will be thawed in the cleanroom at Hoxworth and immediately delivered to CCHMC for infusion.

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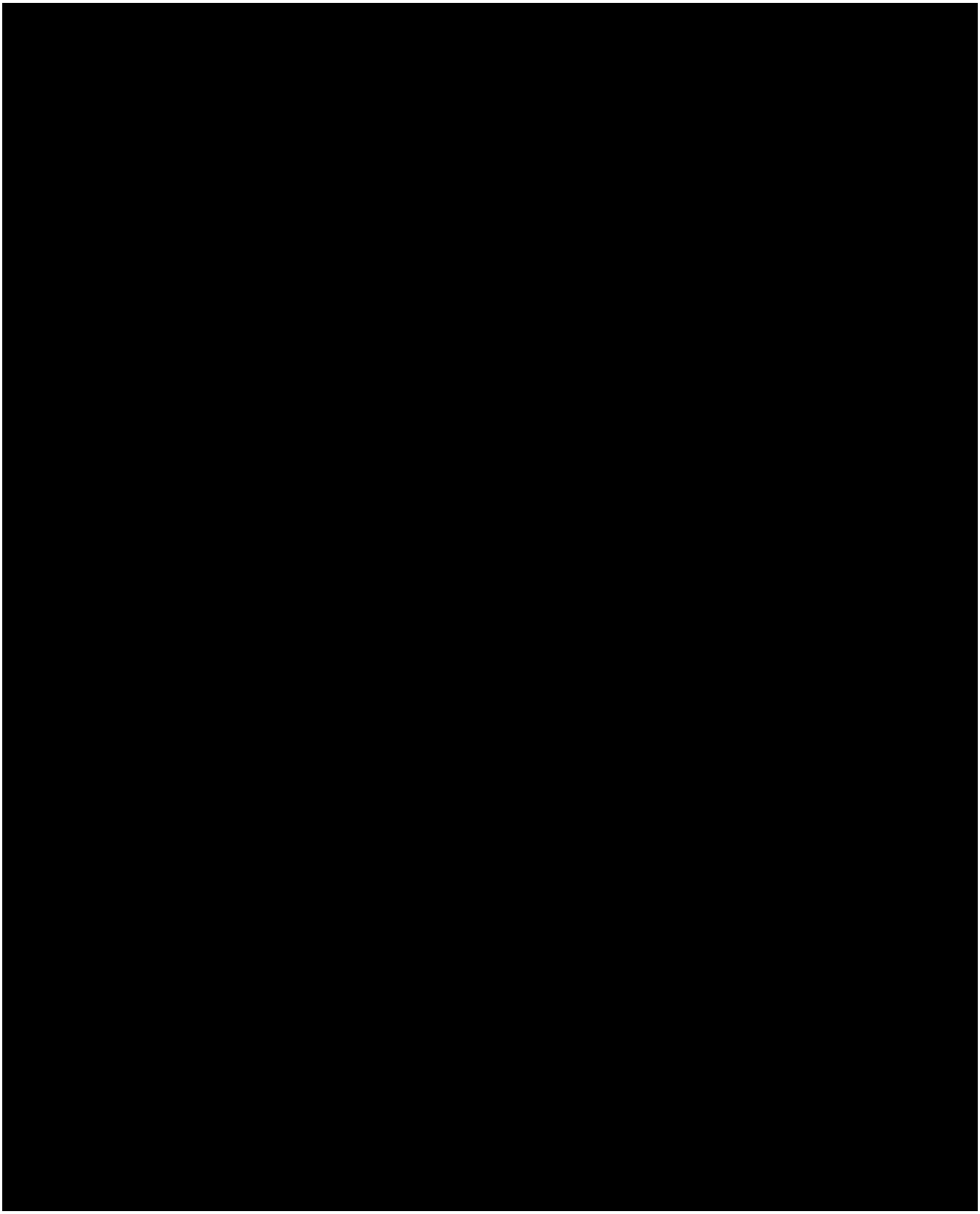
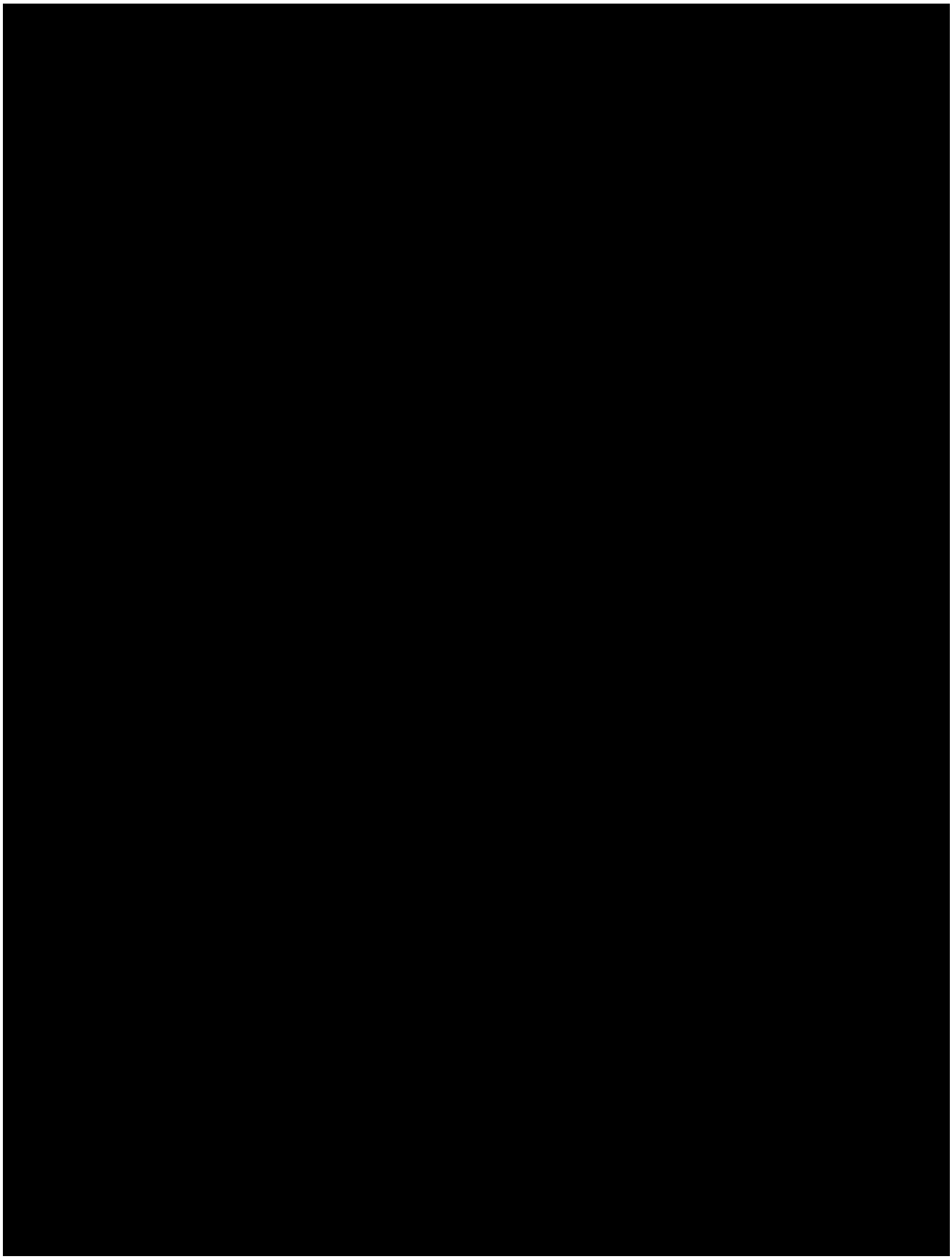




6. PREVIOUS HUMAN EXPERIENCE

Viral infections from Cytomegalovirus (CMV), Epstein-Barr Virus (EBV) Adenovirus (ADV), and BK-virus (BKV) are seen in up to 50% of the HSCT recipients at CCHMC. The intense immunosuppressive regimens necessary during the transplant process cause delayed and often defective immune reconstitution resulting in an increased risk of infection. A retrospective review of 124 pediatric patients undergoing first allogeneic HSCT at CCHMC from January 2009 to December 2010 demonstrated ADV infection in 53% of the patients, EBV infection in 38% and CMV infection in 20% of the patients(Ozdemir, 2012). Conventional anti-viral therapeutic agents are primarily active only against CMV, are associated with significant toxicity, and resistance is frequent; therefore these infections are often fatal for these children. Overall survival was only 70% in this cohort, with viral infection as the cause of death in 10% of the patients. This rate of morbidity and mortality from viral infections is similar to prior published reports at other institutions (O'Reilly, 2007; Curtis, 1999; Zaia, 2002;),thus it is clear that viral infections are a major cause of morbidity and mortality in recipients of allogeneic hematopoietic stem cell transplants (HSCT) from unrelated or mismatched donors.

The majority of the supporting evidence for the use of multi-virus specific T cell immunotherapy also comes from the group at Baylor College of Medicine. They have conducted several small clinical trials in the past 10+ years with either monovalent (anti-EBV), bi-valent (anti-EBV & Adv, or trivalent (anti EBV, Adv, and CMV) VSTs produced as described in this IND. Leen et al first showed in 2006 that infusing monoculture-derived CMV, EBV, and Adenovirus– specific (trivalent) VSTs is a safe and efficient way to restore viral-specific immunity in immunocompromised patients (Leen, 2006). They reported that all individuals with evidence of CMV, EBV, or Adv infection had a decrease in their viral titer and resolution of their disease symptoms that coincided with the expansion of virus-specific VSTs in vivo. This same group also showed that infusions of bivalent cytotoxic T lymphocyte (VST) lines with specificity for EBV and adenovirus can be safely administered to pediatric transplant recipients receiving partially human leukocyte antigen–matched and haploidentical stem cell grafts (n = 13) without inducing graft-versus-host disease (Leen, 2009). None of these 13 high-risk recipients developed EBV-associated lymphoproliferative disease, while 2 of the subjects had resolution of their adenoviral disease. In this particular study, the EBV-specific component of the VSTs expanded in vivo and persisted for more than 12 weeks, but the adenovirus-specific component only expanded in vivo in the presence of concomitant adenoviral infection.

Multivirus-specific T cells generated ex-vivo in this manner, or in a very similar manner, have also been shown by other groups to recognize multiple CMV, EBV, and adenoviral antigens with a broad epitope specificity, and to be able to lyse virus-infected targets in vitro. Micklethwaite, et al used the same AD5f35pp65 vector that Baylor used to transduce dendritic cells and effectively generated anti-CMV T cell clones that significantly improved their patients' anti-CMV immunity post-transplant without any

infusion-related adverse events. (Micklethwaite, 2008). A recent publication by Leen et al (2013) demonstrated the effectiveness of a third party VST bank in treating viral infections. They generated a bank of 32 virus-specific lines from individuals with common HLA polymorphisms immune to CMV, EBV or adenovirus. They were able to demonstrate response rates of between 74-78%, with only 4 responders having a recurrence or progression of their viral infection. No patients experienced toxicity associated VST infusion, and the majority of responders remained virus free at follow-up. A recent follow-up from the TCH group by Tzannou et al (2017) showed that safe and clinically effective VSTs can be generated recognizing CMV, EBV, adenovirus, BKV, and HHV-6 (pentavalent VSTs). In this trial, the cumulative complete or partial response rate was 92%. Other than one patient who developed a fever within 24 hours of infusion there were no immediate toxicities.

None of the above-referenced clinical trials have reported significant toxicities, no increased incidence of GVHD with VST infusions, nor any significant alloreactivity in vitro or in-vivo. A recent safety report from the Baylor group reviewed data from more than 180 recipients receiving more than 380 infusions of a range of antigen-specific and/or engineered T cells and concluded that the treatments are safe (Cruz, 2010). The technology we propose to utilize for generating the virus-specific VST is the same technology that has been used at Baylor College of Medicine, and elsewhere, for several years, in several FDA-approved clinical trials, thus in vitro product efficacy and in vivo product safety have been well demonstrated.

6.1 Safety

As of 12/01/2018 we have enrolled 120 patients on our donor-derived protocol (CCHMC IRB# 2013-2777) with 51 patients receiving 88 infusions. There has been no immediate or long term toxicity associated with the infusions to date, and no patients have developed GVHD related to their VST infusion.

Previous trials at CCHMC with VSTs allowed infusions starting 28 days after stem cell infusion. There have been no infusional toxicities or evidence of acute GVHD developing from the VST infusion . However, in an analysis of viral reactivation amongst this cohort of patients, a significant proportion of viral reactivation occurs between 21 and 28 days after stem cell infusion. Therefore, there appears to be a potential clinical benefit in giving VSTs 21days after stem cell infusion.

7. RESEARCH PLAN

7.1 Overview

This study will be conducted in two steps.

Step 1: Generation of VSTs from consenting donors of eligible donor-recipient pairs to be banked for clinical use (donors are consented under CCHMC protocol #2013-2777)

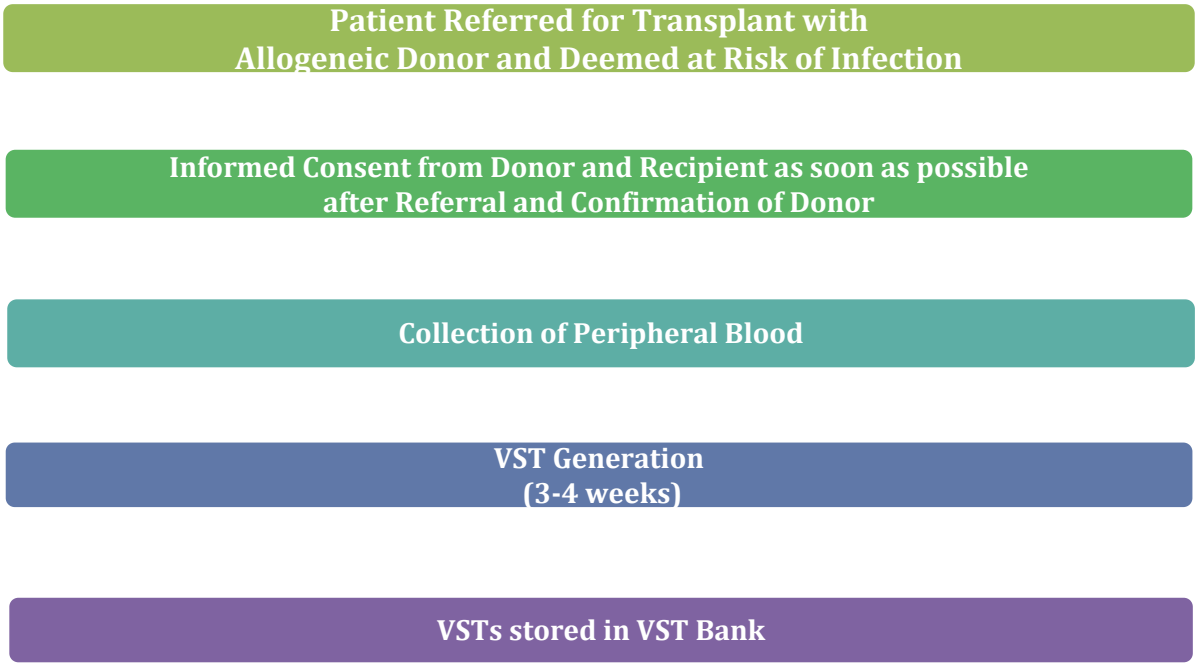
Step 2: Use of quadrivalent VSTs in immunocompromised patients at risk for, but without active evidence of, viral reactivation or disease. If the patient is deemed ineligible for prophylactic infusion due to the presence of viral reactivation or disease, they may be eligible for infusion of the same donor-derived VSTs to be given as a cellular therapy under CCHMC protocol #2013-2777.

In the first step, consenting donors will donate up to 80 mls of blood (or less in smaller donors) and viral specific T-cells will be generated and stored.

In the second step of the study, viral specific VSTs will be infused into recipients without evidence of viral infection or reactivation, as defined below.

7.2 STEP 1: Recipient and Donor Actvities

Generation and Storage of VSTs Offered to all Eligible Donors and Recipients



Both donor and recipient will first consent to the separate VST protocol utilizing donor-derived VSTs for the treatment of viral disease following stem cell transplantation (IRB #2013-2777). Study enrollment on protocol 2013-2777 will be offered to all allogeneic stem cell transplant recipients.

7.3 STEP 2: Recipient Eligibility For Prophylactic VST Reinfusion

7.3.1 Patients will be assessed as they approach Day 21 post stem cell infusion and if they meet the eligibility criteria for a prophylactic VST infusion, the patient's attending physician or a member of the research team will discuss enrolling on this prophylactic VST protocol with the parent/legal guardian or patient if the patient is of age to provide consent.

7.3.2 Patients will be eligible for a prophylactic VST infusion starting at 21 days post stem cell infusion if they have no evidence of viral infection or reactivation defined as below.

- Blood adenovirus PCR ≤1,000
- Blood CMV PCR ≤ 500
- Blood EBV PCR ≤ 9,000
- Plasma BKV PCR ≤ 1,000
- No evidence of invasive adenovirus infection. Adenovirus infection will be 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. Adenovirus disease will be defined as the presence of adenoviral positivity as detected by culture or PCR from more than 2 sites such as stool or blood or urine or nasopharynx.
- No evidence of invasive CMV infection, e.g. pneumonitis, retinitis, colitis.
- No evidence of EBV-associated lymphoproliferation (EBV-LPD) defined as proven EBV-LPD by biopsy or probable EBV-LPD defined as an elevated EBV DNA level in the blood associated with clinical symptoms (adenopathy or fever or masses on imaging) but without biopsy confirmation.
- No evidence of symptomatic BK virus infection, which may include symptomatic hemorrhagic cystitis.

Patients will not receive any anti-viral treatments beyond Acyclovir intended to prevent CMV, EBV, Adenovirus, or BK virus after the VST infusion. Acyclovir is used to prevent HSV1/2 or VZV reactivation post HSCT and is considered the standard of care in patients who are HSV 1 /2 or VZV+. Anti-viral treatment will not be utilized unless there is evidence of clinically harmful evidence of invasive viral disease or clinically significant increase in viral PCR number more than 2 weeks after infusion of VSTs, or the attending physician deems additional anti-viral therapy clinically necessary. Patients who require the initiation of anti-viral medicines or have increasing viral PCR copy number more than 2 weeks after VST infusion will be considered therapeutic failures.

7.3.3 The following inclusion criteria must be met prior to VST infusion

- Recipient must be at least 21 days after stem cell infusion
- Clinical status must allow tapering of steroids to $\leq$ 0.5mg/kg prednisone or other steroid equivalent

7.3.4 Exclusion criteria for infusion of prophylactic VSTs

- Patients who have developed viral infection or reactivation as defined by not meeting the criteria listed in section 7.3.2 will be ineligible for prophylactic infusions of VSTs.
- Active acute GVHD grades II-IV.
- Uncontrolled relapse of malignancy.
- Infusion of ATG or alemtuzumab within 2 weeks of VST infusion. Additionally, in patients who received alemtuzumab as part of their conditioning regimen, alemtuzumab levels will be collected in the second week following stem cell infusion. The level must be less than, or equal to, 0.15 prior to infusion of VSTs. In patients with level greater than 0.15, alemtuzumab levels can be checked serially until a level ≤ 0.15 is obtained. They would become eligible for prophylactic VST infusion at that point if there is still no evidence of viral infection at that time.

7.4 VST Infusion

7.4.1 Patients will receive up to 5 x 10^7 VSTs/m^2. Cells will be infused intravenously using the standard CCHMC SOP for infusion of therapeutic T-cells.

7.4.2 For patients that are given VSTs prophylactically but later develop viral reactivation or disease, they may repeat VST infusion for treatment of viral disease as outlined in CCHMC protocol 2013-2777. Treatment VST infusion cannot be given until 4 weeks following the prophylactic VST infusion.

7.5 Evaluation After VST Infusion

7.5.1 Clinical Assessments

Patients will receive physical examinations daily while inpatient. Following discharge, physical examination will be performed at least weekly until 30 days after last VST infusion.

7.5.2 Laboratory Evaluations

- Complete blood counts weekly until 30 days after last VST infusion
- Comprehensive renal panel with liver function tests weekly until 30 days after last VST infusion
- Viral load monitoring for CMV, Adenovirus, BKV, and EBV weekly until 30 days after the last VST infusion.
- Research samples to assay for the development of viral specific T-cells by Elispot analysis will be collected. Blood (up to 15 mls) will be obtained for

Elispot testing prior to each VST infusion and then weekly for 4 weeks after each VST dose and then monthly as feasible for 12 months after VST infusion.

- If a patient has newly positive viral PCR following VST infusion, research samples (15 mls) to assay for viral specific T-cell persistence by deep sequencing will be collected at the time of viremia and then every 2 weeks for 4 weeks and then monthly for 12 months where feasible.
- A research sample (10 mls) will be collected prior to infusion of VSTs from patients who received ATG as part of their preparative regimen. These will be stored to potentially assay for ATG levels should this cohort of patients have poor efficacy in initial review.
- Urine samples may be collected to assess viral genome and assess the response in viruria to VST infusions prior to each VST infusion and then weekly for 4 weeks after each VST dose and then monthly for 12 months after the last VST infusion. Urine samples will be collected as feasible and/or at the discretion of the PI or designees at these timepoints. If urine samples are not collected at any of these timepoints, it will not be considered a protocol deviation.
- This monitoring plan post VST infusion may be modified to less frequent blood and/or urine samples if the patient is not residing in the local area post VST infusion and collection of research samples poses an undue burden on the VST recipient. Efforts will be made to collect samples locally and have samples sent to CCHMC but, if that is not possible, we will collect research samples in conjunction with concurrent clinical visits.

8. STATISTICAL CONSIDERATIONS

This trial is designed to investigate the safety of donor-derived antiviral VSTs given prophylactically 21 days post stem cell infusion to patients without evidence of active viral disease. Two hundred patients will be enrolled onto the study. It is anticipated that the study will last for 2 years. Previous work in this area predicts that the toxicity of this procedure will be low.

In order to reduce patient risk, the study design includes early termination of the trial in the event of unacceptable infusional toxicity or new onset severe GvHD during the accrual period.

The study will be closed to accrual pending further evaluation if a high rate of severe GVHD (Grade II-IV) develops within 30 days of VST infusion, or if unacceptable rates of infusional toxicity occur. If any death attributable to study procedures occurs, enrollment will be suspended and the SMC, CCHMC IRB and FDA informed.

A total of 200 patients are to be enrolled sequentially into this single arm trial, with the expectation that 122 of these will receive VSTs. The historical rate of grade II-IV GVHD within our institution is 15%. To assess safety of the treatment, a Simon two-stage trial design will be used. In the first stage, 51 patients will be infused with VSTs. If there are more than 15 patients with grade II-IV GVHD, the study will be stopped and the SMC,

CCHMC IRB and FDA will be informed and trial strategy will be reviewed and only restarted with the approval of all regulatory agents. Otherwise, 71 additional patients will be accrued. The safety endpoint will be met if 28 or fewer of the 122 patients have grade II-IV GVHD at any point following infusion.

The primary endpoint of the study is safety of the infusion of viral specific T-cells.

- Infusional toxicity will be monitored using the standard CCHMC therapeutic T-cell infusion SOP. Grades 3 or 4 *attributable* toxicity will be considered unacceptable.
- Patients will be monitored weekly for new onset grades 2-4 GVHD within 30 days of VST infusion.

The following table provides stopping rules for grades 3-4 attributable infusional toxicities. For a given set of patients in the left column, if the number of patients with toxicity equals or exceeds the number in the right column then enrollment will be suspended and the SMC, CCHMC IRB, and FDA informed.

Number of patients	Attributable grades 3-4 infusional toxicity
0-1	1
2 to 7	2
8 to 16	3
16 to 20	4
21 to 27	5
28 to 33	6
34 to 39	7
40 to 45	8
46 to 51	9
52 to 57	10
58 to 62	11
63 to 69	12
70 to 75	13
76 to 80	14
81 to 85	15
86 to 90	16
91 to 95	17
96 to 100	18
101 to 105	19
106 to 110	20
111 to 115	21
116 to 120	22
121 to 125	23

126 to 130	24
131 to 135	25
136 to 140	26
141 to 145	27
146 to 150	28

9. BENEFIT/RISK ANALYSIS

There is the potential for direct benefit for patients undergoing SCT, as evidenced by possible decrease in risk of developing viral infections. However, as this is investigational, the participants may not receive any benefit from this treatment. The information learned from this research study may potentially improve the health care for other patients following SCT in the future.

The study carries a risk of infusional toxicity such as hypertension or fever, and infusion of additional cells could cause graft versus host disease. Based on prior reported experience, it is expected that toxicity of the procedure will be low.

This study is more than minimal risk but with potential of direct benefit to participants.

10. SAFETY ASSESSMENT AND MONITORING

10.1 Adverse Event Monitoring

Safety and tolerability for this study will be assessed according to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE published November 27, 2017). All Grades 3 or 4 *attributable* adverse events and all grade 4 or greater adverse events will be recorded on case report forms. The principal investigator or designee will review each event and assess its relationship to study events to determine whether the event is unrelated or, unlikely, possibly, probably or definitely related to the study therapy. All adverse events that occur from the administration of first VST infusion through 30 days after the last VST infusion will be recorded according to the above criteria. Events occurring greater than 30 days after the last VST infusion will be assessed and recorded only when they are unexpected and determined to be at least possibly related to the VST infusion.

The relationships are categorized as:

- Definite – The adverse event is *clearly* related to the investigational agent(s).
- Probable – The adverse event is *likely* related to the investigational agent(s).
- Possible – The adverse event *may be* related to the investigational agent(s).
- Unlikely – The adverse event is *doubtfully* related to the investigational agent(s).
- Unrelated – The adverse event is *clearly NOT* related to the investigational agent(s).

Determine the prior experience

Expected events are those that have been previously identified as resulting from administration of a stem cell transplant. An adverse event is considered unexpected, for reporting purposes only, when either the type of event or the severity of the event is not listed in the protocol or in the drug package insert for the protocol therapy. The transplant regimen is well known to commonly affect systems such as the hematologic, immunologic, and gastrointestinal systems. These known complications of transplant are listed in the table in Appendix 1 and, for the purposes of this study the listed events occurring at grade 3 or less will not be reported.
Attributable grades 3-4 toxicities will be collected and reviewed by the SMC as well as reported to the CCHMC IRB and the FDA according to each agency's reporting guidelines.

Medical conditions/diseases present before VST infusion are only considered adverse events if they worsen after VST infusion. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

Each sign or symptom reported is documented on the case report form to include the start date, stop date, outcome of event, attribution, and whether the event is expected or unexpected to the study procedures or underlying disease. Adverse Event reporting complies with the current CCHMC IRB policy and applicable federal regulations. If any serious adverse events occur, current guidelines will be followed for expedited reporting to the IRB. Serious Adverse Event reporting complies with the current CCHMC IRB policy and applicable federal regulations.

Expedited reporting requirements for AEs experiences by patients on study:

<table>
<tr><td>Attribution</td><td colspan="2">Grade 3 or 4</td><td>Grade 5</td></tr>
<tr><td></td><td>Unexpected</td><td>Expected</td><td></td></tr>
<tr><td>Unrelated or Unlikely</td><td></td><td></td><td></td></tr>
<tr><td>Possible, Probably, Definite</td><td>Expedited Report</td><td></td><td>Expedited Report</td></tr>
<tr><td colspan="4">Any death that occurs that is attributed (possibly, probably, or definitely) to the agent(s) must be reported according to the instructions above.</td></tr>
</table>

10.2 Study Monitoring and Auditing

Monitoring and auditing procedures will be followed to ensure that the study is conducted, documented, and reported in accordance with the IRB approved protocol, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements of Cincinnati Children's Hospital Medical Center.

Verification of eligibility will be performed and appropriate documentation of informed consent will be documented for all subjects enrolled into the study. All case report forms (CRF) for the first subject enrolled into the study will be monitored for completeness and quality by comparing data in the case report forms to data in the source documents. Thereafter, a minimum of 10% of enrolled subjects' CRFs will be monitored for completeness and quality by comparing data in the case report forms to data in the source documents.

10.3 Data and Safety Monitoring Plan

A safety monitoring committee (SMC) consisting of 3 members, with 2 of those members outside of the Cancer and Blood Disease Institutes, will provide oversight for the conduct of the study. The PI will review study progress with the SMC approximately every 6 months. This includes enrollment/infusion data, as well as review of all reportable adverse events. The SMC will recommend continuation, modification or termination of the study depending on the outcome of their review. All decisions regarding study continuation, modification, or termination will be reported immediately or annually to the CCHMC IRB and FDA, in compliance with current IRB policy and applicable federal regulations.

The SMC will be notified immediately and will review all unanticipated problems involving risk to subjects or others, attributable grades 3-4 toxicities, possibly, probably, or definitely-related and unexpected serious adverse events and all subject deaths associated with the protocol. The SMC will also be notified immediately and conduct a review if a stopping rule is met. Based on the review of any of these events, the SMC will make a recommendation regarding study continuation.

Attributable grades 3-4 toxicities will be collected and reviewed by the SMC as well as reported to the CCHMC IRB and the FDA according to each agency's reporting guidelines.

11. INFORMED CONSENT AND PROTECTION OF HUMAN SUBJECTS

Enrollment will be offered to all eligible recipients. The patient's attending physician or a member of the research team will consult with the parent/legal guardian or patient if the patient is of age to provide consent, to explain the procedures, risks and benefits at the appropriate level of understanding. Opportunity will be given to consider the study and have questions answered. Assent will be obtained from patients between the ages of 11-17 years old.

Informed consent will be obtained prior to study procedures. Only one parent will be required to give permission for enrollment on study.

While we do not plan on targeting non-English speaking patients for enrollment on this study, we do periodically have patients who speak Spanish or Arabic. We will not exclude these patients from this study. We will use a fully translated consent form or a

Spanish or Arabic short form according to the process outlined in the CCHMC Standard Operating Procedures (SOP).

12. PRIVACY AND CONFIDENTIALITY

All documents will be kept in a locked cabinet in the Bone Marrow Transplant Clinical Research Office. Access to the files will be limited to the Principal Investigator and designees. Records will be maintained in a secure database with access is restricted to the Principal Investigator and designees.

Every effort will be made to maintain patient confidentiality. Absolute confidentiality cannot be guaranteed. Patient information may be disclosed if required by law. The FDA, the sponsor and the Cincinnati Children's Hospital Medical Center Institutional Review Board may have access to the records.

13. FUTURE RESEARCH

Data and leftover samples obtained for this study will be stored indefinitely after subject study participation is complete and may be used for future research. Data and samples will be retained unless the participant chooses to withdraw. If the participant withdraws from further study participation, previously collected and stored samples and medical information will either be destroyed or stored for future use based on the participant's request and/or permission. Samples and medical information that have already been distributed to researchers prior to withdraw of consent as well as any data obtained from the distributed samples will not be destroyed.

The data and/or samples may be used by researchers at Cincinnati Children's Hospital Medical Center or at other institutions with IRB approval. Only de-identified data and/or samples will be provided to researchers at other institutions.

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15. APPENDIX 1

Adverse Events Associated with Stem Cell Transplantation That Do NOT Require Reporting

The table overleaf lists expected adverse events associated with stem cell transplantation (based on the Common Terminology Criteria for Adverse Events, version 4.0). Adverse Events listed that are ≤ grade 3 will not be reported, as these are events commonly associated with transplantation. Except in situations described in this section, all other adverse events will be reported to the IRB according to current guidelines.

CATEGORY	AE
BLOOD	Anemia
	Disseminated Intravascular Coagulation
	Febrile Neutropenia
	Hemolysis
	Thrombotic Thrombocytopenia Purpura
Endocrine	Adrenal Insufficiency
	Cushingoid
Gastrointestinal	Colitis
	Constipation
	Diarrhea
	Enterocolitis
	Gastrointestinal Pain
	Malabsorption
	Mucositis
	Nausea
	Pancreatitis
	Vomiting
	Dyspepsia
General	Fever
	Infusion related reaction
	Pain
Hepatobiliary	Cholecystitis
Immune System	Allergic Reaction
	Serum Sickness
Infections and Infestations	Catheter Related Infection
	Enterocolitis Infection
	Lung Infection
	Sinusitis
	Upper respiratory Infection
	Urinary Tract Infection

Investigations	Leukocytopenia
	Lymphocytopenia
	Neutropenia
	Thrombocytopenia
	Weight Gain/Loss
Metabolism and Nutrition	Acidosis
	Alkalosis
	Anorexia
	Dehydration
	Glucose Intolerance
	Iron Overload
Musculoskeletal and Connective Tissue	Generalized Muscle Weakness
Nervous System	Reversible Posterior Leukoencephalopathy Syndrome
Renal and Urinary	Acute Kidney Injury
	Bladder spasm
	Cystitis Noninfective
	Hematuria
	Urinary Frequency
	Urinary Urgency
Reproductive System and Breast	Irregular Menstruation
Respiratory, Thoracic and Mediastinal	Cough
	Pruritis
Vascular	Hypertension
	Purpura
	Petechiae