Scheduled Administration of Virus-Specific T cells for Viral Prophylaxis After Pediatric Allogeneic Stem Cell Transplant

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Abstract:
Infections with double stranded DNA viruses are a significant cause of morbidity and mortality in pediatric patients following allogeneic hematopoietic stem cell transplantation (HSCT). Virus specific T-cell therapy (VSTs) have been shown to be effective treatment for infections with adenovirus, BK virus, cytomegalovirus (CMV), and Epstein-Barr virus (EBV). To date, prophylactic regimens to prevent or mitigate these infections using conventional anti-viral medications provide suboptimal response rates. Here we report on a clinical trial (NCT03883906) performed to assess the feasibility of rapid manufacturing and early infusion of quadrivalent VSTs generated from stem cell donors ("donor derived VSTs") into allogeneic HSCT recipients with minimal or absent viremia. Patients were eligible to receive scheduled VSTs as early as twenty-one days after stem cell infusion. Twenty-three patients received scheduled VSTs. 20/23 had no viremia at the time of infusion while three patients had very low-level BK viremia. Two developed clinically significant graft-versus-host disease, although this incidence was not outside of expected incidence early after HSCT and both were successfully treated with systemic corticosteroids (n=2). Five patients were deemed treatment failures. Three developed subsequent significant viremia/viral disease (n=3). Eighteen patients did not fail treatment, seven of whom did not develop any viremia while 11 developed low-level, self-limited viremia that resolved without further intervention. No infusion reactions occurred. In conclusion, scheduled VSTs appear to be safe and potentially effective at limiting serious complications from viral infections after allogeneic transplantation. A randomized study comparing this scheduled approach to the use of VSTs to treat active viremia is ongoing.

Conflict of interest: COI declared - see note

COI notes: C.M.B is on the advisory board for Cellectis and is on the scientific advisory boards for Catamaran Bio and Mana Therapeutics with stock/or ownership, is on the board of directors for Cabaillet Bio with stock options and has stock in Neximmune and Torque Therapeutics. P.J.H. is a co-founder and on the board of directors of Mana Therapeutics, on the scientific advisory board of Cellevolve, and has intellectual property related to virus-specific T cells. S.M.D has a US pending patent application under review, received research support from Alexion Pharmaceuticals, and consultancy from Novartis (unrelated to this work). The remaining authors declare no competing financial interests.

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Key Points

- Donor-derived VSTs can be manufactured and administered early post-transplant in a prophylactic manner without increased risk of GVHD
- Scheduled infusion of VSTs is associated with few treatment failures and a seeming ability to clear low-level viremia
Abstract:

Infections with double stranded DNA viruses are a significant cause of morbidity and mortality in pediatric patients following allogeneic hematopoietic stem cell transplantation (HSCT). Virus specific T-cell therapy (VSTs) have been shown to be effective treatment for infections with adenovirus, BK virus, cytomegalovirus (CMV), and Epstein-Barr virus (EBV). To date, prophylactic regimens to prevent or mitigate these infections using conventional anti-viral medications provide suboptimal response rates. Here we report on a clinical trial (NCT03883906) performed to assess the feasibility of rapid manufacturing and early infusion of quadrivalent VSTs generated from stem cell donors (“donor derived VSTs”) into allogeneic HSCT recipients with minimal or absent viremia. Patients were eligible to receive scheduled VSTs as early as twenty-one days after stem cell infusion. Twenty-three patients received scheduled VSTs. 20/23 had no viremia at the time of infusion while three patients had very low-level BK viremia. Two developed clinically significant graft-versus-host disease, although this incidence was not outside of expected incidence early after HSCT and both were successfully treated with systemic corticosteroids (n=2). Five patients were deemed treatment failures. Three developed subsequent significant viremia/viral disease (n=3). Eighteen patients did not fail treatment, seven of whom did not develop any viremia while 11 developed low-level, self-limited viremia that resolved without further intervention. No infusion reactions occurred. In conclusion, scheduled VSTs appear to be safe and potentially effective at limiting serious complications from viral infections after allogeneic transplantation. A randomized study comparing this scheduled approach to the use of VSTs to treat active viremia is ongoing.
**Introduction:**

T-lymphocytes are required for the control and eradication of viral infections\(^1\). Infections with double stranded DNA (dsDNA) viruses like cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus (AdV), and BK virus (BKV) are a significant source of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (HSCT) due to the prolonged periods of myelosuppression and immunosuppression needed to promote engraftment\(^2\).\(^5\). Our own institutional prevalence of infection with these viruses by transplant day +100 ranges from 20% to 54% depending on the virus\(^6\). The number of concurrent dsDNA viral infections and the viral load area under the curve are significant risk factors for early and late mortality after HSCT\(^7\). However, both prevention and treatment of viral infections can be difficult. There are currently no FDA approved therapies for the prevention and treatment of AdV and BKV and agents like ganciclovir, foscarnet, and rituximab used for the management of CMV and EBV have high rates of organ toxicity and suboptimal response rates, while often prolonging hospitalizations\(^8\)-\(^12\).

Virus specific T cells derived from a patient’s stem cell donor (donor derived VSTs) can be rapidly manufactured using pools of overlapping viral antigenic peptides, and safely infused for the preemptive treatment of the aforementioned viruses\(^13\),\(^14\). This approach has been effective without an increase in the development of *de novo* graft-vs-host disease (GVHD)\(^15\)-\(^17\). Both autologous and allogeneic VSTs have also been used for prophylaxis against post-transplant lymphoproliferative disease (PTLD), but data on the use of VSTs for prevention or mitigation of viremia are lacking\(^18\). Here we present data from a single arm, phase II trial using scheduled VSTs on or near post-transplant day +21 in patients with no more than clinically insignificant
degrees of viremia and no evidence of invasive viral infection as prophylaxis against significant
viremia or invasive viral disease.

Methods:

Study population and clinical trial

This single arm, phase II study was approved by the Cincinnati Children’s Hospital Medical
Center Institutional Review Board and cleared by the US Food and Drug Administration
(#NCT03883906). All allogeneic HSCT recipients at CCHMC were eligible but required
separate and prior enrollment of both the recipient and donor on a separate study allowing for
generation of donor derived VST (NCT02048332). Patients meeting eligibility criteria and
completing consent were able to receive VST products no sooner than 21 days after stem cell
infusion. Patients received $2 \times 10^7$ VST/m$^2$ as a single infusion. Eligibility for prophylactic VST
infusion required blood AdV PCR < 1 000 copies/mL, CMV PCR < 500 IU/mL, EBV PCR < 9
000 IU/mL, BKV PCR < 1 000 copies/mL, no evidence of invasive CMV or AdV infection, no
evidence of EBV-associated lymphoproliferation, and no evidence of symptomatic BKV such as
hemorrhagic cystitis. Acyclovir at prophylactic dosing for the prevention of herpes simplex virus
and varicella zoster virus was allowed. Clinical status had to allow for tapering of any ongoing
steroids to $\leq 0.5$ mg/kg of prednisone equivalents. Patients were required to be at least two weeks
removed from their last dose of alemtuzumab with a quantified alemtuzumab level of $< 0.15$
$\mu$g/mL$^{19}$. Exclusion criteria included viral infection or reactivation defined by not meeting the
infectious criteria previously mentioned, active acute grade II-IV GVHD, infusion of anti-
thymocyte globulin (ATG) within two weeks of infusion, and uncontrolled relapse of
malignancy. The primary endpoint of the study was to establish the feasibility of producing VSTs and safely infusing as early as 21 days following stem cell transplant without excessive infusional toxicity or an increased incidence of acute GVHD. The study would be feasible if a VST product was successfully manufactured for > 75% of patients. Excessive infusional toxicity was defined as having five attributable grade 3-4 infusional toxicity events in the first 27 patients infused. The historical norms of grade II-IV GVHD at our institution is 15% and the study was initially generated with a Simon two-stage design, with acceptable rates of grade II-IV GVHD being < 29% in the first stage and < 23% in the second stage. The secondary endpoint was clinical efficacy (as defined in “Response Criteria” section below). All infused patients were followed through transplant day +100.

**VST Manufacturing**

VSTs targeting AdV, BKV, CMV, and EBV were generated as previously described\(^\text{20}\). Products were required to meet all release criteria for safety, sterility, and alloreactivity prior to infusion. The percentage of T-cells secreting interferon-gamma in response to pooled viral antigens was performed as previously described\(^\text{20}\).

**Response Criteria**

We anticipated that even with infusion of scheduled VSTs, numerous patients would still develop modest degrees of viremia which would then trigger expansion of VSTs followed by suppression of viremia without the need for anti-viral agents. We therefore expected a reduction in peak levels of viremia and need for antiviral therapy. Thus, thresholds for determining failure of treatment were chosen to be clinically and statistically meaningful and represent levels at which patients would benefit from other pre-emptive anti-viral therapies. Failure cut-offs were
chosen by consensus agreement of senior transplant physicians. The failure cutoffs used in this study, when applied to a prior institutional cohort of 113 consecutive recipients of donor derived VSTs, resulted in a treatment failure rate from viremia of 17%.

Patients were considered treatment failures if any of the following occurred prior to day +100 after transplant: blood AdV PCR > 50,000 copies/mL, BKV PCR > 100,000 copies/mL, CMV PCR > 5,000 IU/mL, EBV PCR > 100,000 copies/mL, evidence of invasive AdV or CMV infection, evidence of EBV-associated lymphoproliferation, evidence of symptomatic hemorrhagic cystitis, initiation of new anti-viral therapy (medication and/or subsequent VST product), or the development of grade III-IV acute GVHD. Surveillance PCR testing for all 4 viruses were checked at least weekly for the first 30 days after infusion and then generally weekly but no less than monthly through transplant day +100. Acute GVHD diagnosis, with stage and grade per Glucksberg criteria were determined by the treating physician in real-time through transplant day +100 and reviewed and confirmed by senior transplant physicians at day 100, and when available, confirmed by biopsy specimens.

**ELISpot**

PBMCs were isolated from the peripheral blood of VST recipients, with samples collected immediately prior to the VST infusion, weekly for the first month after infusion of VSTs, and then monthly through transplant day +100. Interferon-gamma enzyme-linked immunosorbent assay (ELISpot) was then performed using these samples to assess for the presence and proliferation of anti-viral T-cells in the peripheral blood over time as previously described.
Results:

Patient Characteristics

The study period began in March 2019 with the last enrollment in October 2020. During this time, 138 patients at CCHMC underwent first allogeneic HSCT. Of those, 30 enrolled in the study (21.7%). The most common reason for non-enrollment was a lack of VST product which occurred in 44 patients (31.9%). Reasons to not have product included donor declining blood draw, donor and/or recipient residing in a country that did not allow procurement of research samples, manufacturing failure, recipient of cord blood graft, and delays in donor sample collection for VST manufacturing leading to delays in product generation. Other reasons for non-enrollment included disqualifying levels of viremia/viral infection (these patients were eligible for treatment infusion of VST- 23/138, 16.7%), primary physician preference due to clinical instability or other cause (16/138, 10.6%), patient/family decline (12/138, 8.7%), and primary graft failure (3/138, 2.2%). One haploidentical HSCT recipient received prophylactic VSTs and developed skin GVHD requiring treatment with systemic steroids, and as a result all recipients of haploidentical transplant were subsequently excluded from the study to remove significant HLA mismatch as a potential confounding risk factor and to allow for greater cohort homogeneity.

During the study period, 10/138 (7.2%) patients undergoing first allogeneic HSCT were recipients of haploidentical transplantation and were thus ineligible.

Of the 30 patients who enrolled, 23/30 ultimately received prophylactic infusions. Reasons for not receiving infusions were acute medical decompensation prior to VST infusion (n=2), development of disqualifying levels of viremia between consent and infusion (n=3; all subsequently received donor derived VSTs on a preemptive treatment protocol), and patient/family withdrawal prior to infusion (n=2). Demographic information on the 23 infused
patients is shown in Table 1. 20/23 had no viremia at time of infusion while 3 patients had <
1000 copies of BKV. Of the five patients who received alemtuzumab prior to HSCT, the median
alemtuzumab level prior to VST infusion was 0.05 µg/mL (range 0-0.12). One patient was
receiving corticosteroids (for immunosuppressive purposes) at the time of infusion at a dose of
0.37 mg/kg/day of prednisone. No patients had a prior history of GVHD.

**VST product characteristics**

All patients received VST products manufactured from their stem cell donor. VSTs were
generated from donors fully matched with their recipients in 20 cases and mismatched at one
HLA allele in 2 cases. One patient received a haploidentical graft that matched at 8/10 HLA
alleles. EBV and CMV serologies were known for all donors and showed prior infection in 12/23
(52.2%) and 18/23 (78.3%) of donors, respectively. T-cells with activity against EBV and CMV
were not able to be generated from donors who were seronegative for those specific viruses. Of
the 13 patients who had donors that were seronegative for EBV and/or CMV, 7 were seropositive
for the respective virus pre-transplant while 6 were seronegative. Donor BKV and AdV
serologies were not tested due to the ubiquitous nature of these viruses. The fold expansion of
cells in culture was 16.70 fold (range 10.1-35.5) with a median post-thaw viability of 84% (range
71-90%) (Figure 1A, B). CD4+ cells out-numbered CD8+ cells in 21/23 (91.3%) products. The
median CD4+:CD8+ ratio was 3.0 (range 0.7-21.4) (Figure 1C). The median percentage of
intracellular interferon-gamma positive T-cells by flow cytometry was 0.33% for CMV (range
0.00-10.11), 3.98% for ADV (range 1.43-14.91), 0.21% for BKV (range 0.00-5.78) and 0.10%
for EBV (range 0-3.6). Amongst seropositive donors, the median for CMV was 2.29% (range
0.02-10.11) and for EBV was 0.24% (range 0.00-3.6)
Prophylactic VST administration early after HSCT is feasible with an acceptable safety and tolerability profile

116/138 (84.1%) patients enrolled on the study for generation of donor derived VSTs. Only 11/116 of those patients (9.5%) and 11/138 of the entire cohort (8.0%) did not have a product available due to manufacturing issues. An additional 4 patients had VSTs available but not until after day +21 leading to a total of 15/138 patients (10.9%) who did not have cells available by this early time point, although this study did allow enrollment of patients with delayed VSTs. Of note, 15 additional patients had products that met all safety and release criteria and thus were available for preemptive treatment of viremia but not acceptable for this study due to manufacture during a three-month audit of laboratory procedures. These products were not considered manufacturing failures. As > 75% of patients had a product manufactured successfully, the feasibility endpoint was met. Between the 11 patients who did not have a product available due to manufacturing and the 23 patients excluded due to early viral reactivation, a total of 34/138 patients (24.6%) were unable to enroll due to objective pre-defined criteria. As a result 75.4% of patients were considered for inclusion. The median day of infusion was transplant day +23 (range day +21-+40). Median absolute lymphocyte count (ALC) at infusion was 380 (range 130-1550) (Figure 2A). 11/23 patients received VSTs on the first day of eligibility. All patients received the target cell dose of 2 x 10^7 VST/m^2. The most common reasons for delay were due to scheduling/logistics of infusion (n=7), delay in consent process (n =2), delay in VST generation (n=2), primary physician preference (n=1), and awaiting alemtuzumab clearance (n=1). Only three infusions occurred on or after day+30: one due to manufacturing delays (infused on day +30), one due to primary physician preference, and one
due to awaiting alemtuzumab clearance. The median ALC 30 days after infusion was 560 (range 120-3210); ALC was increased in 17/23 (73.9%) (Figure 2A). 2/6 (33.3%) patients with decreased ALC received systemic corticosteroids within the first 30 days after VST infusion which may explain the lack of lymphocyte expansion.

Scheduled VSTs were given early in the post-transplant period. As a result, we anticipated seeing some GVHD in our cohort but did not expect the incidence to be above our historical norm. In a cohort of 200 consecutive allogeneic transplant recipients at our institution between 2015-2018, the rate of grade III-IV GVHD within 100 days of transplant was 14.5% and the rate of grade III-IV GVHD was 7%. Two cases of GVHD occurred within 30 days of VST infusion and were considered possibly attributable to VSTs: one patient with grade II skin GVHD and one patient with grade III-IV skin and GI GVHD. GVHD developed on transplant day +40 (14 days after VST infusion) and day +38 (17 days after VST infusion), respectively.

Three additional patients developed GVHD more than 30 days after infusion, all low grade (grade I skin only in two patients, grade I skin and grade II GI in 1 patient) and were considered not attributable to VSTs. The incidence of grade II-IV and III-IV GVHD by transplant day +100 in this cohort was 3/23 (13.0) and 1/23 (4.3%) which are in line with our prior experience of the incidence of GVHD in this population. The study was initially designed to infuse 122 patients and the pre-specified safety endpoint was 28 or fewer patients (23.0%) with grade II-IV GVHD at any point following infusion. Since only 13% of patients in this initial cohort had grade II-IV GVHD, we opted to close this study and proceed with a successor randomized study comparing scheduled VSTs with preemptive VSTs which is now ongoing.
There were no cases of cytokine release syndrome. Transplant-associated thrombotic microangiopathy was seen in two cases (8.7%) diagnosed at 6 weeks and 18 months after VSTs respectively. Both were treated with eculizumab. No infusion reactions occurred.

_Treatment failure occurs in a minority of patients infused with prophylactic VSTs_

Five of the 23 patients infused with VSTs were classified as treatment failures (21.7%). Patients who failed treatment are described in table 2. One patient failed due to the development of EBV viremia requiring treatment with rituximab that arose after receiving steroids for the management of grade II skin GVHD. This patient was the recipient of a haploidentical transplant and haploidentical VSTs. One patient failed due to the development grade III-IV skin and gut GVHD occurring within 30 days of infusion; this patient was also treated with steroids and subsequently also developed significant ADV viremia and mild EBV viremia. Two patients were treatment failures due to peak viremia levels meeting protocol failure threshold, one for CMV and one for EBV (peak levels of 9,400 and 528,917 respectively). In both cases, patients cleared the viremia with subsequent antiviral therapy (valganciclovir for CMV and rituximab followed by third party VSTs for EBV). The final treatment failure had symptomatic BKV dysuria; this did not require further anti-viral therapy and ultimately cleared but the patient did require an admission for pain control. Of the three patients who failed therapy due to either CMV or EBV viremia, donor serology was positive for the respective virus in all three cases. Of the four patients who were treatment failures due to viremia or viral disease, two patients (Patient #1 and #23) had VST products with anti-viral activity greater than the median value for that virus within the entire cohort, while two patients (#9 and #20) had products with activity that was lower than the median.
Four treated patients died, all after day +100. Two of these deaths were in patients who were treatment failures; one of bacterial infection 18 months post-infusion, and one from multi-organ failure in the context of GVHD and bacteremia at 5.5 months post-infusion. Two were in patients who were not treatment failures, both of whom died from relapse of leukemia (4 months and 15 months post-infusion respectively). Median post-transplant day at death was day +347 (range 143-675). All other patients are currently alive, although two have had relapse of malignancy at six- and ten- months post infusion, respectively.

Eleven of 18 of the non-failure patients developed viremia from at least one virus (Table 3). Three patients had low level CMV viremia (median peak viremia of 1647, range 1112-2329) which in all cases resolved without additional intervention. Five patients had low level BKV virema (median peak viremia of 623, range 500-5513), which in all cases resolved without intervention. Six patients had low level EBV viremia (median 4564, range 500-46751). None of these patients required EBV-directed therapy and viremia resolved entirely in two patients while there was residual viremia of 9,206 in one patient and < 200 in three patients at day +100, which then subsequently cleared without intervention. Donor serology was positive for the respective virus in the eight patients who had any EBV or CMV viremia (2 patients with CMV, 5 patients with EBV, 1 patient with both). Seven of the 18 non-treatment failures (38.9%) did not develop any viremia by transplant day +100 (Table 3).

ATG was used during conditioning in 3/5 (60.0%) treatment failures but was used in only 3/18 (16.7%) non-failing patients. Alemtuzumab was given in conditioning to 1/5 (20.0%) treatment failures and in 4/18 (22.2%) non-failing patients, but unlike in the ATG setting, antibody clearance was monitored and ensured in these cases. Abatacept was given after transplantation to 2/5 (40.0%) of treatment failures and 6/18 (33.3%) of non-failing patients.
Anti-viral T-cells are present in peripheral blood of VST recipients and poised to expand upon detection of viral ligand

Interferon-gamma ELISpot assays were performed for all VST recipients on weekly samples of PBMCs drawn for the first month and then monthly thereafter when possible.

Of the seven patients who never developed viremia, increased viral directed T-cells were detected in the peripheral blood in six, starting from a baseline median of 14 SFC/4 x 10^5 PBMC (range 1.4-69) to a median peak of 49.8 SFC/4 x 10^5 PBMC (range 29-315) (Figure 2B). Increase above the baseline was seen in 16/17 (94.1%) of the ELISpot assays performed in this cohort. One patient, who had a donor who was EBV and CMV negative, was an outlier with very high baseline T-cell numbers against ADV and BKV (> 500) that subsequent decreased to zero at all other time points and was excluded from analysis due to concern for an inaccurate baseline study.

In the 11 patients with 15 distinct viremias that improved without intervention, increase in viral directed T-cell count above the baseline was seen on ELISpot in all 15 instances, going from a median baseline of 7.0 SFC/4 x 10^5 PBMC (range 0-271.0) to a median peak of 177.0 SFC/4 x 10^5 PBMC (range 8.0-1517.0) (Figure 2C). Representative examples showing the kinetics of viral clearance in association with corresponding increase in anti-viral T cells are shown in Figure 2D.

Two treatment failures (one patient with CMV viremia that resolved after adding valganciclovir and one patient with symptomatic BK cystitis that subsequently resolved without intervention but required hospitalization for pain control), had increases in spot number by ELISpot with
peaks of 276.2 and 14.0 SFC/4 x 10^5 PBMC respectively. Sample size is small but there were no obvious qualitative differences in the kinetics of T-cell expansion or elevation in these patients as compared to non-failure patients. ELISpots from the three other treatment failures had confounding factors that make interpretation difficult; in two cases the patients received lympholytic steroids and in one case the viremia started after the research samples had been collected.

**Discussion**

In this feasibility study we report the prophylactic administration of scheduled quadrivalent donor derived VSTs to 23 pediatric patients with no or very minimal viremia early after allogeneic HSCT. Most patients either never developed viremia or developed low level viremia that cleared without subsequent viral directed therapy. VSTs were infused at a median transplant day +23, a time during which there are few or no endogenous functional T-cells. We believe our data support the hypothesis that scheduled VSTs from mostly fully HLA matched stem cell donors can persist in recipients, poised to expand upon detection of viral ligand. Accordingly, this approach may allow for decreased complications from dsDNA viruses after HSCT.

Efficacy outcomes on this study were promising although this was not the primary endpoint and need to be cautiously interpreted in this single arm study. More definitive efficacy conclusions require a randomized study which is now ongoing. In this study, seven patients (30.4%) never developed viremia by day +100 after transplant. It is notable that all seven of these patients had a 10/10 HLA match with their donors and 4/7 received grafts from siblings so this is generally a population at lower risk for viral infection after HSCT\textsuperscript{22,23}. There were 11 patients who did develop low-level viremia but were able to clear the infection without further intervention.

Interestingly, in each case of viremia there was a corresponding increase in anti-viral T-cells on
ELISpot following the initial detection of viremia by PCR. As this occurred early after transplant while the ALC is low, we presume that this increase in interferon-gamma secreting T-cells is primarily made up of the donor derived VSTs, although we did not definitively demonstrate this. These data suggest that donor derived VST infused in the absence of viral ligand persist and can expand upon encountering viral antigen (Figure 2D). T-cell receptor sequencing could further demonstrate whether T cells arose from VSTs or the stem cell graft, and this will be an area of future study.

The goal of this study was to infuse as close to 21 days after stem cell infusion as possible as generally this is around or before most viremias occur. 44 patients (31.9%) of the cohort were not eligible due to lack of VST product, however 11/44 were due to manufacturing failures and 15/44 were due to otherwise acceptable products not used during a lab audit. As a result, only 18/138 (13.0%) lacked a product for reasons outside of our control. With expected decreases in manufacturing failures over time, we believe the number of patients who will not have a product will be acceptably low although this will be answered on an ongoing successor randomized study. Despite infusing T-cell products at a time when acute GVHD often first develops, only two patients developed GVHD during the first 30 days after infusion and only one was grade III-IV. Importantly, the historical rates of grade II-IV and grade III-IV GVHD at our institution in a cohort of 200 consecutive patients was 14.5% and 7% respectively, making the incidence of GVHD seen in this study in line with prior institutional norms. Of note, the protocol was amended to make recipients of haploidentical transplants ineligible due to GVHD in one recipient. Haploidentical transplants make up a small percentage of HSCT at our institution but is increasing throughout the field as a whole. While our successor trial excludes patients with a match of less than 9/10, to definitively determine the risk to recipients of
haploidentical transplant we plan to open a separate study for solely those patients with stringent stopping rules surrounding the development of GVHD.

VST therapy, whether from an individual’s stem cell donor or from a 3rd party donor, has been shown to be a safe and effective approach for the pre-emptive treatment of these viral infections, even in patients who have failed conventional anti-viral therapy^{16,20-33}. VSTs have largely been used in the treatment rather than the prevention of viral infections. Medical prophylaxis regimens are incompletely effective at preventing viral reactivation and often result in undesired toxicities. Letermovir has shown promise for prophylaxis in CMV positive patients although is currently only FDA approved in adults^{34}. As a result, trialing VST for prevention is rational. One small study of twelve recipients showed VSTs could be safely infused as early as transplant day +2 (with a median for their cohort of day +13) with tolerable side effect profile^{35}. CMV reactivation was seen in 50% of patients on this study although all were in the context of steroid therapy. Another recent study demonstrated prophylactic delivery of VSTs to 11 adult patients but at a median infusion time of day +37 (range of day +28-76)^{36}. Additional anti-viral treatment was only required in patients who received steroids although 4 patients did develop grade III-IV GVHD. The data from this study supports our hypothesis that scheduled donor derived VSTs limit viral infections and obviate the need for anti-viral medications after HSCT. Both prior studies enrolled only adult patients and to our knowledge our cohort represents the first study investigating prophylactic VSTs in pediatric patients. We believe this study support the use of scheduled VSTs, not necessarily to decrease the percentage of patients who develop viremia, but instead to limit the degree of viremia and need for conventional anti-viral medications. Currently, we have an ongoing randomized study comparing the prophylactic approach to the use of VSTs for the treatment of viremia and if confirmed, this approach could become a new
standard of care for viral prophylaxis. If pediatric clinical trials of letermovir show similar outcomes as adult studies, adding letermovir in combination with scheduled VSTs would be testable and reasonable.

This open label study was limited by the lack of a control arm. However, it is promising that only 3/23 (13.0%) patients received additional anti-viral therapy. Although it is difficult to make direct statistical comparisons with a historical cohort due to possible selection biases, it is notable that in a cohort of 200 consecutive allogeneic transplant recipients at our institution from the immediate pre-VST implementation period, 46% of patients required treatment with conventional anti-viral therapy.

A limitation of this study is the small sample size, with only 23 patients receiving a scheduled VST product, accounting for 16.7% of all allogeneic transplant recipients during the study period and 19.8% of patients enrolled on the protocol to generate donor derived VSTs. It is reassuring that only 8.0% of patients did not receive an infusion due to a lack of available product, suggesting enrollment can be optimized on successor studies through changes in eligibility criteria. The small sample size was in part driven by excluding patients with ongoing viremia or viral infection. Our ongoing successor randomized trial will attempt to overcome this confounding factor by including patients with pre-existing viremia to determine if VSTs given at a scheduled early time point of day +21 have improvements with regard to viral complications and peak viremia as compared to the standard of care strategy of receiving treatment VSTs at the providers discretion. It is also likely that there will always be a proportion of recipients without donor derived VSTs. Third-party VSTs are an important option in these cases, at least for treatment, if not prophylaxis. One additional weakness is that since the VSTs are not tagged, we cannot definitively show that expanded populations of T-cells originate from the VST. Since
VSTs are given at a time of profound lymphopenia, we presume the expansion seen on ELISpot are of donor origin, but future studies will incorporate T-cell receptor sequencing to further establish this point.

In summary, the administration of scheduled VSTs as a preventative measure in patients with no or minimal viremia was safe with GVHD rates in line with our institutional standard. These data, if supported by additional studies, raise the prospect of an important change in standard of transplant care, with most recipients likely to benefit from this strategy.
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Authorship: J.D.R., S.M.D, A.S.N, and M.S.G. designed the study, performed research, and wrote the manuscript. X.Z., G.P, L.R, performed functional studies and analysis. A.L. contributed to statistical design of the study. C.L. and T.L performed and supervised manufacturing and characterization of VSTs. D.L and J.F. performed data collection and analysis. J.A.C, S.T., C.D., J.W., M.W., C.M.B, P.J.H, and M.D.K provided vital conceptual insights for study design, assisted with study subject accrual and data collection, and provided scientific support. All authors reviewed and edited the manuscript.

Conflict of interest disclosure: C.M.B is on the advisory board for Cellectis and is on the scientific advisory boards for Catamaran Bio and Mana Therapeutics with stock/or ownership, is on the board of directors for Caballeta Bio with stock options and has stock in Neximmune and Torque Therapeutics. P.J.H. is a co-founder and on the board of directors of Mana Therapeutics, on the scientific advisory board of Cellevolve, and has intellectual property related to virus-specific T cells. S.M.D has a US pending patent application under review, received research
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References:

1. Walker LJ, Sewell AK, Klenerman P. T cell sensitivity and the outcome of viral infection. *Clin Exp Immunol.* 2010;159(3):245-255.

2. Styczynski J, Czyzewski K, Wysocki M, et al. Increased risk of infections and infection-related mortality in children undergoing haematopoietic stem cell transplantation compared to conventional anticancer therapy: a multicentre nationwide study. *Clin Microbiol Infect.* 2016;22(2):179 e171-179 e110.

3. Admiraal R, de Koning CCH, Lindemans CA, et al. Viral reactivations and associated outcomes in the context of immune reconstitution after pediatric hematopoietic cell transplantation. *J Allergy Clin Immunol.* 2017;140(6):1643-1650 e1649.

4. Koskenvuo M, Rahiala J, Sadeghi M, et al. Viremic co-infections in children with allogeneic haematopoietic stem cell transplantation are predominated by human polyomaviruses. *Infect Dis (Lond).* 2017;49(1):35-41.

5. Huang ZY, Chien P, Indik ZK, Schreiber AD. Human platelet FcgammaRIIA and phagocytes in immune-complex clearance. *Mol Immunol.* 2011;48(4):691-696.

6. Ozdemir N, Jodele S, Myers KC, et al. Prevalence of Viral Infections in Children Undergoing First Allogenic Hematopoietic Stem Cell Transplantation: Two Year Single Center Experience. *Biol Blood Marrow Transplant.* 2012;18(2):S315-S316.

7. Hill JA, Mayer BT, Xie H, et al. The cumulative burden of double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. *Blood.* 2017;129(16):2316-2325.

8. Lugthart G, Oomen MA, Jol-van der Zijde CM, et al. The effect of cidofovir on adenovirus plasma DNA levels in stem cell transplantation recipients without T cell reconstitution. *Biol Blood Marrow Transplant.* 2015;21(2):293-299.

9. Emery V, Zuckerman M, Jackson G, et al. Management of cytomegalovirus infection in haemopoietic stem cell transplantation. *Br J Haematol.* 2013;162(1):25-39.

10. Patriarca F, Medeot M, Isola M, et al. Prognostic factors and outcome of Epstein-Barr virus DNAemia in high-risk recipients of allogeneic stem cell transplantation treated with preemptive rituximab. *Transpl Infect Dis.* 2013;15(3):259-267.

11. Wy Ip W, Qasim W. Management of adenovirus in children after allogeneic hematopoietic stem cell transplantation. *Adv Hematol.* 2013;2013:176418.

12. Jacobsen T, Sifontis N. Drug interactions and toxicities associated with the antiviral management of cytomegalovirus infection. *Am J Health Syst Pharm.* 2010;67(17):1417-1425.

13. Baugh KA, Tzannou I, Leen AM. Infusion of cytotoxic T lymphocytes for the treatment of viral infections in hematopoietic stem cell transplant patients. *Curr Opin Infect Dis.* 2018;31(4):292-300.

14. Barrett AJ, Prockop S, Bollard CM. Virus-Specific T Cells: Broadening Applicability. *Biol Blood Marrow Transplant.* 2018;24(1):13-18.

15. Feuchtinger T, Matthes-Martin S, Richard C, et al. Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. *Brit J Haematol.* 2006;134(1):64-76.

16. Gerdemann U, Katari UL, Papadopoulou A, et al. Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. *Mol Ther.* 2013;21(11):2113-2121.

17. Leen AM, Christin A, Myers GD, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood.* 2009;114(19):4283-4292.
18. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood.* 2010;115(5):925-935.

19. Marsh RA, Lane A, Mehta PA, et al. Alemtuzumab levels impact acute GVHD, mixed chimerism, and lymphocyte recovery following alemtuzumab, fludarabine, and melphalan RIC HCT. *Blood.* 2016;127(4):503-512.

20. Nelson AS, Heyenbruch D, Rubinstein JD, et al. Virus-specific T-cell therapy to treat BK polyomavirus infection in bone marrow and solid organ transplant recipients. *Blood Adv.* 2020;4(22):5745-5754.

21. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation.* 1974;18(4):295-304.

22. Copelan OR, Sanikommu SR, Trivedi JS, et al. Higher Incidence of Hemorrhagic Cystitis Following Haploidentical Related Donor Transplantation Compared with Matched Related Donor Transplantation. *Biol Blood Marrow Transplant.* 2019;25(4):785-790.

23. Srinivasan A, Wang C, Srivastava DK, et al. Timeline, epidemiology, and risk factors for bacterial, fungal, and viral infections in children and adolescents after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2013;19(1):94-101.

24. Keller MD, Darko S, Lang H, et al. T-cell receptor sequencing demonstrates persistence of virus-specific T cells after antiviral immunotherapy. *Br J Haematol.* 2019;187(2):206-218.

25. Hiwarkar P, Gaspar HB, Gilmour K, et al. Impact of viral reactivations in the era of pre-emptive antiviral drug therapy following allogeneic haematopoietic SCT in paediatric recipients. *Bone Marrow Transplant.* 2013;48(6):803-808.

26. Duver F, Weissbrich B, Eyrich M, Wolfl M, Schlegel PG, Wiegering V. Viral reactivations following hematopoietic stem cell transplantation in pediatric patients - A single center 11-year analysis. *Plos One.* 2020;15(2).

27. Yanik G, Levine JE, Ratanaathathorn V, Dunn R, Ferrara J, Hutchinson RJ. Tacrolimus (FK506) and methotrexate as prophylaxis for acute graft-versus-host disease in pediatric allogeneic stem cell transplantation. *Bone Marrow Transplant.* 2000;26(2):161-167.

28. MacMillan ML, Holtan SG, Rashidi A, DeFor TE, Blazar BR, Weisdorf DJ. Pediatric acute GVHD: clinical phenotype and response to upfront steroids. *Bone Marrow Transplant.* 2020;55(1):165-171.

29. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood.* 2013;121(26):5113-5123.

30. Clancy LE, Blyth E, Simms RM, et al. Cytomegalovirus-Specific Cytotoxic T Lymphocytes Can Be Efficiently Expanded from Granulocyte Colony-Stimulating Factor-Mobilized Hemopoietic Progenitor Cell Products Ex Vivo and Safely Transferred to Stem Cell Transplantation Recipients to Facilitate Immune Reconstitution. *Biol Blood Marrow Tr.* 2013;19(5):725-734.

31. Tzannou I, Papadopoulou A, Naik S, et al. Off-the-Shelf Virus-Specific T Cells to Treat BK Virus, Human Herpesvirus 6, Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections After Allogeneic Hematopoietic Stem-Cell Transplantation. *J Clin Oncol.* 2017;35(31):3547-3557.

32. Qian C, Wang Y, Reppel L, et al. Viral-specific T-cell transfer from HSCT donor for the treatment of viral infections or diseases after HSCT. *Bone Marrow Transplant.* 2018;53(2):114-122.

33. Withers B, Clancy L, Burgess J, et al. Establishment and Operation of a Third-Party Virus-Specific T Cell Bank within an Allogeneic Stem Cell Transplant Program. *Biol Blood Marrow Transplant.* 2018;24(12):2433-2442.
34. Marty FM, Ljungman P, Chemaly RF, et al. Letervir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation. *N Engl J Med.* 2017;377(25):2433-2444.

35. Muranski P, Davies SI, Ito S, et al. Very Early Adoptive Transfer of Ex Vivo Generated Multi-Virus Specific T Cells Is a Safe Strategy for Prevention of Viral Infection after Allogeneic T Cell Depleted Stem Cell Transplantation. *Blood.* 2018;132.

36. Gottlieb DJ, Clancy LE, Withers B, et al. Prophylactic antigen-specific T-cells targeting seven viral and fungal pathogens after allogeneic haemopoietic stem cell transplant. *Clin Transl Immunology.* 2021;10(3):e1249.
Table 1: Characteristics of patients infused with prophylactic virus specific T-cells

| Characteristic                                      | No. (%) of Infused Patients (N = 23) |
|-----------------------------------------------------|--------------------------------------|
| Gender                                              |                                      |
| Male                                                | 11 (47.8)                            |
| Female                                              | 12 (52.2)                            |
| Race/Ethnicity                                      |                                      |
| Hispanic                                            | 2 (8.7)                              |
| Black or African American                           | 1 (4.3)                              |
| White                                               | 13 (56.5)                            |
| Other                                               | 7 (30.4)                             |
| Median age at infusion, years (range)               | 10.3 (0.7-22.9)                      |
| Transplantation type                                |                                      |
| Matched unrelated donor                             | 13 (56.5)                            |
| Matched related donor                               | 7 (30.4)                             |
| Mismatched unrelated donor                          | 2 (8.7)                              |
| Haploidentical donor                                | 1 (4.3)                              |
| Stem cell source                                    |                                      |
| Bone marrow                                         | 11 (47.8)                            |
| Peripheral blood                                    | 12 (52.2)                            |
| Conditioning regimen                                |                                      |
| Myeloablative                                       | 16 (69.6)                            |
| Reduced intensity                                   | 7 (30.4)                             |
| Reason for transplantation                          |                                      |
| Malignancy                                          | 12 (52.2)                            |
| Immunodeficiency                                    | 2 (8.7)                              |
| Non-malignant hematology                            | 2 (8.7)                              |
| Bone marrow failure syndrome                        | 7 (30.4)                             |
| Serotherapy received during conditioning             |                                      |
| Alemtuzumab                                         | 5 (21.7)                             |
| ATG                                                 | 6 (26.1)                             |
| GVHD prophylaxis                                    |                                      |
| Calcineurin inhibitor containing regimen            | 14 (60.9)                            |
| Ex vivo T-cell depletion                             | 9 (39.1)                             |
| Abatacept                                           | 8 (34.7)                             |

Abbreviations: ATG, anti-thymocyte globulin; GVHD, graft-vs-host disease; HSCT, hematopoietic stem cell transplantation
Table 2: Characteristics of scheduled VST treatment failures

| Patient Number | Indication for transplant | Conditioning intensity and graft | GVHD prophylaxis | EBV and CMV serostatus (Donor/Recipient) | Viral load prior to VST infusion | Transplant day at infusion | Criteria for treatment failure | Peak viral load by 100 days after transplant | Additional antiviral therapy (post-transplant day therapy started) | Status at last follow-up |
|----------------|---------------------------|----------------------------------|------------------|------------------------------------------|-------------------------------|------------------------|-----------------------------|---------------------------------------------|-------------------------------------------------|-----------------------|
| 1              | ALL, CR1                  | MAC Haploidentical PBSC          | T-cell depletion | CMV: -/+ EBV: +/- Plasma BK 911           | 27                            | EBV viremia             | 536, 198 (EBV)              | Rituximab (day +94)               | None                                            | Dead, sepsis, day 675 |
| 9              | FA                        | MAC MUD PBSC                    | T-cell depletion | CMV: +/- EBV: +/- Plasma BK 927           | 21                            | Symptomatic BK viruria  | 11,837 (BKV)                | None                                         | Alive                                           |                       |
| 20             | SDS                       | RIC MUD Marrow                  | CSA, MMF, abatacept | CMV: -/+ indeterminate EBV: +/-          | N/A                           | EBV viremia             | 528,917 (EBV)              | Rituximab (day +82), 3rd party VST (day +83) | None                                            |                       |
| 22             | β-thalassemia             | MAC MUD Marrow                  | Tacrolimus, MMF, abatacept | CMV: +/- EBV: +/-                    | N/A                           | CMV viremia             | 9,400 (CMV)                | Valganclovir (day +57)            | None                                            |                       |
| 26             | NK lymphohistiocytopenia disorder | RIC MUD Marrow                 | Tacrolimus, MMF | CMV: +/- EBV: +/-                     | N/A                           | Grade III-IV GVHD      | 308,723 (ADV)              | Cidofovir (day +98)             | None                                            | Dead, MOF, day 199 |

Abbreviations:
- ADV, adenovirus
- ALL, acute lymphoblastic leukemia
- BKV, BK virus
- CMV, cytomegalovirus
- CSA, cyclosporine A
- CR1, first complete remission
- EBV, Epstein-Barr virus
- FA, Fanconi anemia
- GVHD, graft-vs-host disease
- MAC, myeloablative conditioning
- MMF, mycophenolate mofetil
- MOF, multi-organ failure
- MUD, matched unrelated donor
- NK, natural killer cell
- PBSC, peripheral blood stem cells
- RIC, reduced intensity conditioning
- SDS, Shwachman-Diamond syndrome
- VST, virus-specific T-cell
| Patient Number | Indication for transplant | Conditioning Intensit y and graft | GVHD prophylaxis | EBV and CMV serostatus (Donor/Recipient) | Vira l load prior to VST infusion | Transplant day at infus ion | Transplant day at first viremi a | Peak viral load by 100 days after transplant | Viral load at 100 days after transplant | Transplant day when viremia cleared | Status at last follow up |
|----------------|--------------------------|----------------------------------|------------------|----------------------------------------|-------------------------------|-----------------------------|-------------------------------|---------------------------------------------|---------------------------------------------|--------------------------------|---------------------------------|
| 5              | AML                      | MAC MUD PBSC                     | CSA, MTX, abatacept | CMV: +/- EBV: +/-                      | N/A                           | 22                          | 26, CMV                      | 1112                          | None                          | 71                             | Alive                           |
| 6              | MPA L                    | MAC 9/10 Unrelated PBSC          | CSA, MMF, abatacept | CMV: +/- EBV: +/-                      | N/A                           | 25                          | 50, EBV                      | 7480                          | <200                          | N/A                            | Alive, relapse after 16 months |
| 10             | ALL                      | MAC 9/10 Unrelated BM            | CSA, MMF, abatacept | CMV: +/- EBV: +/-                      | N/A                           | 22                          | 34, BKV                      | 623                           | None                          | 62                             | Alive                           |
| 12             | MDS                      | MAC 8/10 Unrelated PBSC          | T-cell depleti on  | CMV: +/- EBV: +/-                      | N/A                           | 40                          | 48, CMV 83, EBV               | 2329, CMV 1150, EBV          | None                          | 69, CMV 89, EBV                | Deceased, relapse, day 494    |
| 14             | FA                       | MAC MRD PBSC                     | T-cell depleti on  | CMV: +/- EBV: +/-                      | N/A                           | 21                          | 45, CMV                      | 1647                          | None                          | 66                             | Alive                           |
| 15             | MDS                      | MAC MUD Marrow                   | CSA, MMF, abatacept | CMV: +/- EBV: +/-                      | N/A                           | 28                          | 40, BKV 47, EBV              | 2742, BK 8456, EBV           | None, BK < 200, EBV           | 92, BKV N/A, EBV              | Alive                           |
| 17             | FA                       | MAC MUD PBSC                     | T-cell depleti on  | CMV: +/- EBV: +/-                      | N/A                           | 21                          | 45, EBV                      | 46,751                        | 9,206                         | N/A                            | Alive                           |
| 24             | Severe aplastic anemia   | RIC MUD PBSC                     | T-cell depleti on  | CMV: +/- EBV: +/-                      | N/A                           | 23                          | 64, BKV                      | <500                          | None                          | 74                             | Alive                           |
| 25             | FA                       | MAC MUD PBSC                     | T-cell depleti on  | CMV: +/- EBV: +/-                      | BK, 713                       | 29                          | 19, BKV                      | 5514                          | None                          | 100                            | Alive                           |
| 29             | ALL                      | MAC MRD PBSC                     | CSA, MMF          | CMV: +/- EBV: +/-                      | N/A                           | 21                          | 48, EBV                      | 4348                          | None                          | 97                             | Alive                           |
| 30             | ALL                      | MAC MRD                          | CSA, MMF          | CMV: +/- EBV: +/-                      | N/A                           | 23                          | 76, EBV                      | 4039, EBV < 200, EBV          | None                          | N/A, EBV                       | Alive                           |
Table 3: Characteristics of patients with viremia that did not require additional antiviral therapy

| Abbreviations: ADV, adenovirus; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia, BKV, BK virus; CMV, cytomegalovirus; CSA, cyclosporine A; EBV, Epstein-Barr virus; FA, Fanconi anemia; GVHD, graft-vs-host disease; HSCT, hematopoietic stem cell transplant; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MPAL, mixed phenotypic acute leukemia; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; PBSC, peripheral blood stem cell; VST, virus specific T-cell |

Table 4 Characteristics of patients who never developed viremia
| Patient Number | Indication for transplant | Conditioning Intensity | GVHD prophylaxis | EBV and CMV serostatus (Donor/Recipient) | Serotherapy | Transplant day at infusion | Status at last follow up |
|----------------|---------------------------|------------------------|------------------|----------------------------------------|-------------|---------------------------|------------------------|
| 2              | AML                       | MAC MRD Marrow         | Tacrolimus, MTX  | CMV: -/+ EBV: +/-                      | None        | 21                        | Alive                  |
| 3              | Severe aplastic anemia    | RIC MUD PBSC           | T-cell depletion | CMV: -/+ EBV: +/-                      | Alemtuzumab | 22                        | Alive                  |
| 8              | HLH                       | RIC MUD PBSC           | CSA, prednisone  | CMV: +/- EBV: +/-                      | Alemtuzumab | 39                        | Alive                  |
| 13             | ALL                       | MAC MUD Marrow         | CSA, MMF, abatacept | CMV: -/+ EBV: +/-                  | None        | 24                        | Dead, relapse day 143  |
| 16             | Hgb SD                    | RIC MRD Marrow         | CSA, MTX, abatacept | CMV: +/- EBV: -/-                  | Alemtuzumab | 22                        | Alive                  |
| 19             | ALL                       | MAC MRD Marrow         | CSA, MMF         | CMV: +/- EBV: -/+                    | None        | 25                        | Alive                  |
| 28             | AML                       | RIC MRD PBSC           | T-cell depletion | CMV: +/- EBV: +/-                    | Ex-vivo depletion only | 24                        | Alive, relapse 6 months after HSCT |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CSA, cyclosporine; A, GVHD, graft-vs-host disease; Hgb, hemoglobin; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplant; MAC, myeloablative conditioning; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; PBSC, peripheral blood stem cell; VST, virus specific T-cell

Figure Legends:
Figure 1: Pre-clinical testing of VST products infused into patients on this study. A. Fold expansion of VSTs in culture. B. Percentage of viable cells after thawing of cryopreserved products. C. Ratio of CD4:CD8 T-cells in each product. Lines in all panels represent median values.

Figure 2: Increase in anti-viral T-cells in peripheral blood of patients after receiving scheduled VSTs. A. Absolute lymphocyte counts at infusion (pre-) and 30 days after VST infusion (post-) in all recipients. B, C Baseline (pre-infusion) and peak (any point post-infusion) quantitated anti-viral T-cells as determined by interferon-gamma ELISpot in recipients without any viremia and with viremia that cleared without intervention respectively. D. Representative examples of ELISpots from non-treatment failure patients with viremia that resolved with corresponding curves showing kinetics of viremia.
Figure 1

A. Fold expansion

B. % post-thaw viable cells

C. CD4:CD8 ratio
