A Phase II Trial of Primary Transplant Donor Derived CMVpp65 specific T-cells for The Treatment of CMV Infection or Persistent CMV Viremia after Allogeneic Hematopoietic Stem Cell Transplantation

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Susan Prockop, M.D.	Pediatrics, BMT
Co-Principal Investigator(s)/Department:	Parastoo Dahi, M.D.	Medicine, BMT
Investigator(s)/Department:	Jaap Jan Boelens, M.D., Ph.D. Farid Boulad, M.D. Maria Cancio, MD Kevin Curran, M.D. Nancy Kernan, MD Andrew Kung, MD Andromachi Scaradavou, M.D. Barbara Spitzer, M.D. Ekaterina Doubrovina, MD, Ph.D. Juliet Barker, M.B.B.S. Christina Cho, M.D. David Chung, M.D., Ph.D Sergio Giralt, M.D. Boglarka Gyurkocza, M.D. Alan Hanash, M.D. Katharine Hsu, M.D., Ph.D. Ann Jakubowski, M.D., Ph.D. Oscar Lahoud, MD Heather Landau, M.D. Esperanza Papadopoulos, M.D. Geneva Papanicolaou, M.D. Jonathan Peled, M.D. Miguel-Angel Perales, M.D. Ioannis Politikos, M.D. Doris Ponce, M.D. James Scott, M.D., Ph.D. Michael Scordo, M.D. Brian Shaffer, M.D. Craig Sauter, M.D. Gunjan Shah, M.D. Melody Smith, M.D. Roni Tamari, M.D. Marcel R.M. van den Brink, M.D., Ph.D James Young, M.D	Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Infectious Disease Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT Medicine, BMT
Consenting Professional(s)/Department:	Jaap Jan Boelens, M.D., Ph.D. Farid Boulad, M.D. Nancy Kernan, MD Susan Prockop, M.D. Andromachi Scaradavou, M.D. Barbara Spitzer, M.D. Kevin Curran, M.D.	Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT Pediatrics, BMT

	Maria Cancio, MD	Pediatrics, BMT
	Andrew Kung, MD	Pediatrics, BMT
	Juliet Barker, M.B.B.S.	Medicine, BMT
	Christina Cho, M.D.	Medicine, BMT
	David Chung, M.D., Ph.D.	Medicine, BMT
	Parastoo Dahi, M.D. Sergio	Medicine, BMT
	Giralt, M.D. Boglarka	Medicine, BMT
	Gyurkocza, M.D.	Medicine, BMT
	Alan Hanash, M.D	Medicine, BMT
	Katharine Hsu, M.D., Ph.D.	Medicine, BMT
	Ann Jakubowski, M.D., Ph.D.	Medicine, BMT
	Oscar Lahoud, MD	Medicine, BMT
	Heather Landau, M.D.	Medicine, BMT
	Esperanza Papadopoulos, M.D	Medicine, BMT
	Jonathan Peled, M.D.	Medicine, BMT
	Miguel-Angel Perales, M.D.	Medicine, BMT
	Ioannis Politikos, M.D.	Medicine, BMT
	Doris Ponce, M.D.	Medicine, BMT
	James Scott, M.D., Ph.D.	Medicine, BMT
	Michael Scordo, M.D.	Medicine, BMT
	Craig Sauter, M.D.	Medicine, BMT
	Brian Shaffer, M.D.	Medicine, BMT
	Gunjan Shah, M.D.	Medicine, BMT
	Melody Smith, M.D.	Medicine, BMT
	Roni Tamari, M.D.	Medicine, BMT
	Marcel R.M. van den Brink, M.D., Ph.D	Medicine, BMT
	James Young, M.D	Medicine, BMT
	For Donor and Patient Assessment Consents ONLY:	
	Nicole Lestrange, NP	Medicine, BMT
	Victoria Szenes, NP	Pediatrics, BMT
	Melissa Varda, RN	Pediatrics, BMT
	Jennifer Bieler, RN	Pediatrics, BMT
	Adlynn Parado, RN	Pediatrics, BMT

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a single institution phase II trial designed to assess the therapeutic activity of CMVpp65 specific T-cells (CMVpp65-CTL) for the treatment of CMV infection or persistent CMV viremia after allogeneic hematopoietic stem cell transplantation. T-cells for infusion will be generated from the seropositive transplant donor by sensitization using autologous dendritic cells loaded with a pool of overlapping synthetic peptides spanning the sequence of CMVpp65.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objectives:

1. To assess in a single arm Phase II trial, the therapeutic activity of transplant donor derived CMVpp65-CTL for the treatment of clinically overt CMV infection or CMV viremia that is persistent despite 2 weeks of treatment with antiviral agents such as Ganciclovir and /or foscarnet.
2. Determine the risk of inducing GvHD or increasing its severity by adoptive transfer of transplant donor derived CMVpp65-CTL.

Secondary Objectives:

1. Determine the individual efficacy of transplant donor derived CMVpp65-CTLs in decreasing viral load by quantitating the level of CMV DNA in the blood of patients following adoptive transfer.
2. Examine the dynamics of in-vivo proliferation and survival of adoptively transferred donor derived CMVpp65-CTLs by sequentially quantitating the alterations in frequency and specificity after adoptive transfer.
3. Determine the degree to which treatment with CMVpp65-CTLs can eliminate or reduce the exposure to antiviral agents such as ganciclovir and fosrcarnet and thus reduce the associated morbidity from organ specific toxicity of antiviral agents.

3.0 BACKGROUND AND RATIONALE

Reactivation of latent cytomegalovirus infections remains a major cause of morbidity and mortality following allogeneic hematopoietic stem cell transplants (HSCT). Although prophylactic or preemptive treatment with ganciclovir or foscarnet has reduced the incidence and mortality of early CMV infections in HLA-matched recipients (1,2), in 6-30% of cases, treatment may be hampered or cannot be sustained due to complicating myelosuppression or nephrotoxicity (3,4). Prolonged anti-viral treatment may also delay the recovery of virus-specific immune responses, leading to a significant incidence of late onset disease. After 3 months of treatment with ganciclovir, late CMV infection was reported by Boeckh et al. in 17.8% of 146 seropositive recipients of HLA-matched unmodified marrow transplants (5,6). These late infections were lethal in 46% of cases, and were asscociated with persistent deficiencies in absolute numbers of $CD4^+$ and $CD8^+$ T-cells and CMV-specific T-cells (5,6). In our own study of 255 recipients of HLA-matched $CD34^+E^-$ T-cell depleted HSCT who did not receive post transplant immunosuppression, the cumulative incidence of CMV reactivation among seropositive patients was similar to that reported following unmodified HSCT (58% vs 40-60%), and only 12% developed clinically overt disease (7). Similar to findings reported

for unmodified allo HSCTs, deficiencies of $CD4^+$ and $CD8^+$ T-cells were significantly associated with disease (6,7).

CMV infections are particularly dangerous in seropositive recipients of HLA non-identical HSCT. For example, while the overall mortality of CMV infections in recipients of HLA-matched HSCT has been reduced to 3-5%, (9,10) mortality rates of 13-16% have been reported among recipients of HLA-haplotype disparate Tcell depleted grafts (11). Similarly, for seropositive recipients of HLA partially matched immunologically naïve cord blood transplants, the cumulative incidence of clinically significant CMV disease has been reported to be as high as 29%, with mortality rates of 6% to 9% despite treatment with antiviral drugs (12-14).

Adoptive Immunotherapy for CMV

Healthy seropositive individuals contain high frequencies of CMV and EBV-specific CD4 [+] and CD8 [+] T-cells, ranging from 0.4 - 3% of the total T-cell population, that play a critical role in controlling in these latent viral infections (8). The potential of virus-specific T-cells to control CMV and EBV infections was initially suggested by the close correlation between reconstitution of CMV and EBV virus-specific CD8+ cytotoxic T-cells and the potential of allogeneic HCT recipients to clear CMV and EBV-induced disease (15-17). Subsequently, we and others have demonstrated that adoptive transfer of in vitro generated transplant donor-derived virus specific T-cells can clear life-threatening clinical CMV infections and induce durable remissions of clonal EBV-induced lymphomas emerging after allogeneic HSCT (18-30). Our group provided the first demonstration of the potential of DLI to treat EBV lymphomas in 1994 (25). Since then, we have been conducting a trial of transplant donor derived EBV-specific T-cells in the treatment of biopsy proven monoclonal EBV lymphomas post allogeneic HSCT. In our initial experience, three weekly infusions of 10^6 EBVCTL/Kg recipient weight have induced durable CRs in 13/19 (68%) patients treated (22,23).

Riddell et al.(26) were the first to demonstrate the feasibility and clinical efficacy of CMV specific T-cells for the prophylaxis and treatment of CMV reactivation in post transplant patients. In this study, 14 patients with persistent CMV viremia or those at high risk were treated with 4 escalating weekly doses of CMV-specific CD8+ T-cell clones (10^7, 3x 10^7, 10^8 and $10^9/m^2$) using donor peripheral blood mononuclear cells (PBMC) sensitized with autologous CMV infected fibroblasts. All 14 patients responded by clearing the viremia, and none of the patients developed GvHD. Subsequent clinical trials have demonstrated clearance of CMV viremia in recipients of HLA matched or one antigen-mismatched related or unrelated donor HSCT treated for persistent CMV viremia unresponsive to antiviral drugs with infusion of $5 \times 10^5 - 1 \times 10^6$ CMV specific T-cells/kg or 10^3- 10^4 CMVpp65 responsive $IFN\gamma^+$ T-cells/kg (21,27-29). Thus studies by Peggs et al (21), and Einsele et al (27), used 1 x 10^5/kg (Peggs) or 3.3 x 10^5/kg(Einsele) CMV specific T-cells for treating CMV viremia or infections in recipients of matched related or unrelated HSCT and demonstrated successful clearance of CMV viremia and reconstitution of CMV specific immune reponses in 19/24 treated patients, with no grade 2 or greater GvHD. Micklethwaite et al (28) prophylactically infused 5 x 10^5CMVpp65-CTL/kg for prevention of CMV infections in 12 recipients of HLA matched or 1 allele mismatched related or unrelated HSCT with only 4/12 patients developing transient CMV viremia and no increased incidence of GvHD in this prophylaxis trial. Feuchtinger et al (29) treated 18 patients after allo-SCT from HLA–mismatched/ haploidentical or HLA–matched unrelated donors with 2 x 10^4 $IFN\gamma^+$ polyclonal CMVpp65-CTL /kg, 15/18 cleared CMV and there was no reported

GvHD in any of the cases.

T-cells used in these studies were sensitized against CMV using PBMC or autologous dendritic cells (DCs) loaded with viral lysate (21,27), or autologous DCs transduced with CMVpp65 (28), A third approach employs the isolation of interferon-γ^+ T-cells CliniMACS using IFNγ capture microbeads (Miltenyi Biotec) (29).

Our phase I trial of transplant donor-derived CMVpp65 peptide specific T-cells (05-065) has completed accrual and required follow up and is being readied for publication. Overall, 8/10 evaluable patients that had failed ganciclovir and/or foscarnet durably, cleared CMV viremia after single doses of 0.5, 1 or 2 x 10^6 CMVpp65-CTLs/kg; all 46 patients treated with 3 weekly doses of 1 x 10^6/kg CMVpp65 CTLs cleared CMV viremia. Elimination of CMV was consistently correlated with increments in circulating CMVpp65-CTL of the same epitope specificity and Vβ usage as the infused CMVpp65-CTL. Detectable numbers of CMVpp65 CTLs were sustained for 2-4 months. Acute toxicities were rare, no patient developed de novo GvHD.

We now propose a single-armed Phase II trial to evaluate transplant donor derived derived CMVpp65CTL in the treatment of CMV infections. This study will allow us to evaluate the ability of this treatment modality to prevent or limit the use of anti-viral drugs with their associated toxicities in patients treated with donor derived CMVpp65 T-cells.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a single-arm non-randomized single institution phase 2 trial, designed to evaluate the therapeutic activity of CMVpp65-CTLs generated from seropositive HSCT donors when adoptively transferred into transplant recipients with persistent CMV infection or viremia.

Patients eligible for this trial will be consenting recipients of related or unrelated HSCT who have an active CMV infection or persistent CMV viremia for ≥ 2 weeks despite treatment with anti-viral agents or who cannot be maintained on anti-viral therapy due to treatment related toxicity.

Patients will be treated with CMVpp65-CTLs derived from their transplant donor. These will be patients with CMV seropositive transplant donors who have previously provided leukocytes for generation of CMVpp65-CTL and for whom such CMVpp65-CTL are available.

CTLs for infusion will be generated by repeated sensitization of donor derived T-cells over 28 days using autologous dendritic cells (DCs) or EBV transformed B cells loaded with a pool of 138 synthetic peptides spanning the sequence of CMVpp65. (confer section 5.0 and appended Standard Operating Procedures).

Concurrent Therapy : Antivirals and Immunosuppression

All patients receiving anti-viral drugs may be maintained at their current doses during treatment with CTLs. Patients can continue treatment with rapamycin and calcineurin inhibitors. However, steroid

doses must be adjusted so that: Patients are receiving no more than 0.3 mg/kg of prednisone or its equivalent.

Characterization and Monitoring of CMV Infections and T-cell Responses:

Prior to and at specific intervals following infusion of CMVpp65-CTL, patients wil be monitored for : 1) vital signs and clinical and radiologic responses at sites of CMV infection, 2) level of circulating CMV DNA by PCR, and levels of CMVpp65-CTL in the blood as measured by quantitation of CTL precursor frequencies, CMVpp65-specific IFNγ+ T-cells (35), or epitope specific-T-cells binding HLA-peptide tetramers (36). The CMVpp65-CTL infused will also be characterized as to TCR Vβ usage and the sequence of immunodominant T-cells defined so as to be able to track disposition, and longevity of these T-cells post infusion. Patients will also be monitored for any toxicities as described in section 11.0.

4.3 Intervention

The T-cells to be infused will be selected based on criteria mentioned in section 4.0 from our bank of GMP grade CMVpp65-CTL. T-cells will be administered by bolus intravenous infusion. In this phase II trial, patients will be treated at doses of 1 x 10^6 CMVpp65-CTL/kg/dose/week for 3 weeks. Patients will be observed for the following 3 weeks. Additional 3 week courses of CMVpp65-CTL may be administered if levels of CMV DNA in blood are still detectable despite disease stabilization or improvement.

The rationale for the doses of T-cells proposed for treatment stems not only from our Phase I trial but also from our own studies of quantitating doses of unselected T-cells required to induce GvHD and the doses of EBV –CTLs required to induce regressions of EBV lymphomas following allogeneic HSCT. Our studies of the incidence of GvHD in recipients of T-cell depleted matched or 1 allele mismatched HSCT suggest that a dose of ≥ 10^5 allo cytotoxic T-cell (CTLp)/kg, as quantitated by limiting dilution analyses, represents the threshold dose for development of grade II or greater GvHD (39). In-vitro sensitization of T-cells against CMV or EBV for a period of 28 days results in preferential proliferation of antigen specific T-cells with simultaneous depletion of alloreactive T-cells by at least 100 fold (34). Therefore, in the doses of CMVpp65-CTL proposed for treatment in this trial (1x 10^6/kg x 3 T-cells /kg), the doses of potentially alloreactive T-cells administered would be estimated to be below the GvHD threshold. In practice, the CMVpp65CTL after this culture period do not contain detectable allocytoxic activity against patient or fully allogeneic PHA blasts. Indeed, this lack of alloreactivity is a release criterion that must be met to use the cells for treatment.

Our clinical experience also confirms this. In our Phase I trial of CMVpp65CTL, no patient developed GVHD. Similarly, none of the 19 patients with EBV lymphomas in our study developed GvHD when treated with EBV specific T-cells sensitized for 4 weeks with irradiated autologous EBV BLCLs at doses ranging from 15-150 x 10^5 T-cells/kg (21). Rooney et al (19), also observed no cases of GvHD among 39 patients treated prophylactically with transplant donor derived EBV specific T-cells generated by a similar technique over a dose range of 3.3 – 33 x 10^5 T-cells /kg.

Thus, based on our own experience, as well as data from published series, the risk of GvHD is expected to be very low, and the doses of infused CMVpp65-CTLs will provide enough numbers of CMV reactive T-cells to produce effective responses against CMV infection.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Donors of CMV-Specific T-cells

The CMV-specific T-cell lines to be used for treatment in this protocol and to be maintained cryopreserved under GMP conditions in the Adoptive Immune Cell Therapy Facility at MSKCC, will be derived fromthe groups listed below:

1. Normal, HLA-typed, CMV-seropositive donors of allogeneic hematopoietic progenitor cell allografts, who previously consented and gave blood or leukocytes to establish CMV-specific T-cells to be used to treat a CMV infection were it to develop in the patient to whom they donated a hematopoietic cell graft. Once that patient has reconstituted immune function and is no longer at risk for CMV, the donors will be asked for consent, in writing, for the use of these T-cells to treat CMV in a patient other than the patient for whom they were originally intended.

2. Normal, HLA typed, CMV-seropositive related and unrelated hematopoietic cell transplant donors who consent to provide blood or blood white cells to make CMV-specific T-cells for treatment of CMV in the recipient of their transplant will also be asked whether the CMV-specific T cell line generated can be used for another patient, at such time as the transplant recipient is no longer at risk for this complication.

3. Normal, HLA typed, CMV-seropositive volunteer blood donors whose T cells may add to the HLA diversity of the T cells available in the Adoptive Immune Cell Therapy Bank.

Adequate health for donation as determined by institutional (related donor) or NMDP (unrelated donor) guidelines. Normal donors will be evaluated for evidence of prior sensitization to CMV by CMV serology. They will also be typed for HLA A, B, C, DR and DQ.

Clinical studies for donors are obtained within 1 week of blood donation and include CBC with differential and platelet count. Results of tests must be within a range that would not preclude donating blood or undergoing leukapheresis. Infectious disease markers will be performed as per each department's guidelines or at the discretion of the treating attending.

For prospective transplant donors, an informed consent will be obtained and up to 2 blood samples will be collected:

i. An initial donation of 25 ml blood (heparin ACD) to be used for establishing a B cell line transformed with the B95.8 laboratory strain of EBV. This EBV+ B cell line (EBV BLCL) will be used as an antigen presenting cell for T-cell sensitization. When loaded with the pool of CMVpp65 peptides, the EBV BLCL efficiently sensitize T-cells from the same donors against CMV as well as EBV.

   The establishment of the EBVBLCL line requires 4-5 weeks of in-vitro culture. Therefore, for recipients of unrelated or HLA disparate HSCT who are at high risk for severe CMV infection in the first 2-3 months, this blood sample should be collected prior to the donation of the hematopoietic progenitor cell transplant whenever possible.

ii. A donation of a single standard 2 blood volume leukapheresis collected in standard ACD anticoagulant is required for the isolation of adequate numbers of T-cells and feeder cells required for T-cell sensitization and extended culture in vitro. If a leukapheresis is not possible, a unit of whole blood is acceptable.

In addition to providing for isolation of T-cells for sensitization with CMVpp65 peptide loaded APCs, this blood donation also provides autologous feeder cells essential to sustain T-cell growth without the risk of stimulating the growth of alloreactive T-cells capable of inducing GvHD.

This donation of a leukapheresis or a unit of blood is obtained from unrelated HSCT donors.

In order to limit the number of donations required of any donor, each donor will be informed of, and asked to consent to, two other potential uses of the blood cells in the donation. They include

1. The making of T-cells reactive against Epstein Barr virus to treat treat lethal lymphomas caused by this virus in HSCT recipients.
2. The making of T-cells reactive against a protein called WT-1, that is expressed by malignant blood cells.

Such T-cells could be used under separate protocols, to treat EBV associated diseases (IRB 95-024) and /or to treat or prevent leukemia recurrence (IRB 07-055) in the patient receiving the HSCT.

In the event that the T-cells isolated and generated from a given donor are not used by the intended HSCT recipient, if the donor so consents, they will be cryopreserved and maintained under GMP conditions in the Adoptive Immune cell Therapy Facility at MSKCC.
These stored T-cells may be used for the treatment of other patients with CMV or EBV infections/malignancies (IRB 11-130) that express HLA alleles shared by the donor.

Generation of Antigen Presenting cells (Dendritic cells and EBV BLCL) and Peptide Loading of APCs

The DCs to be used for T-cell sensitization are generated from PBMCs isolated from blood leucocytes by Ficoll-Hypaque gradient separation and enriched for monocytes by adherence to plastic sterile tissue culture plates or by positive selection with CD14 clinical grade microbeads (miltenyi) if the frozen/thawed PBMC is used. The monocytes are then re-suspended at a concentration of 1 x 10^6 cells/ml in RPMI supplemented with 1% heat inactivated autologous serum (if available) or prescreened heat inactivated human AB serum (Gemini) clinical grade *GMCSF* (2000 IU/ml) and *interleukin-4* (1000 IU/ml). The cultures are be supplemented with these cytokines on days 2 and 4 in fresh medium as indicated by cell growth. On days 5 or 6, 5 ng/ml of *TNFα*, 2.5 mg/ml of *IL-1β*, 75 µg/ml of *IL-6* and 0.5µg/ml *PGE-2* are be added for 48 hours to induce maturation of dendritic cells. Thereafter, the dendritic cells are be washed, adjusted to a concentration of 1 x

10^6/ml, and loaded with the pool (25µg complete pool/ml) of overlapping CMV-pp65 synthetic peptides (Invitrogen, Boston Massachussetts) in serum free medium at 37ºC for 3 hours and used for T-cell sensitization as described below.

EBV transformed B cells are be generated as described previously (33), adjusted to a concentration of 1 x 10^6/ml, and loaded with 25µg CMVpp65 peptide pool/ml in serum free medium at 37ºC for 3 hours and used for T-cell sensitization.

Generation of CMVpp65 Specific T-cells

T-cells will be generated as described in published reports and detailed in the appendices (34). Briefly, CD3+ enriched T-cell fractions are initially isolated by ficoll-hypaque gradient separation of peripheral blood leucocytes. Thereafter, monocytes are depleted by adherence to the plastic tissue culture flasks or by immunoadsorption using clinical grade CD14 immunomagnetic microbeads (Miltenyi Biotechnology, Gmbh, Gladback, Germany) if done from frozen/thawed PBMC. The $CD56^+$ NK^+ cells and B cells are then removed by immunoadsorption to immunomagnetic beads coated with clinical grade mouse monoclonal antibody specific for CD56 and CD19 respectively (CliniMACS CD56 reagent, Miltenyi Biotechnology, Gmbh, Gladback, Germany). Aliquots of 1 x 10^6 T-cells /ml will then be stimulated with 0.5 x 10^5/ml autologous irradiated monocyte- derived DC or with EBV BLCL previously loaded with a pool of 138 synthetic overlapping pentadecapeptides spanning the sequence of CMV pp65. The stimulated T-cells will then be cultured in modified Yssel's medium supplemented with 5% pre-screened heat inactivated human AB serum in sterile flasks at 37ºC, with 5% CO2 in air. The culture will be re-stimulated with the peptide loaded DCs or EBV BLCL at an effector to target ratio of 10:1 at 7 day intervals for 21-28 days. Clinical grade IL-2 (Chiron) will be added to a concentration of 10-80 IU/ml every 3 days, beginning on day 7 and IL-15 weekly at the dose of 10ng/ml (stimulation with DCs does not potentiate significant expansion of T-cells. The earlier initiation of cytokine stimulation at higher doses permits generation of higher numbers of T-cells to meet the dose requirements in the protocol. IL-15 has been described as a potent stimulator of T-cell expansion in-vitro. After 21-28 days in culture, the T-cells will be harvested, counted and tested for potency, specificity and lack of alloreactivity, microbiological sterility, and for levels of endotoxin (confer appended Standard operating procedures). If a sufficient number of T-cells is obtained, the T-cells will then be aliquoted into sterile vials in calculated doses tested as detailed below and cryopreserved for subsequent administration to the recipient of the donor's HSCT or for a third party recipient if consented for treatment of CMV infection or persistent viremia. If additional numbers of T-cells are required, the pre-generated CMVpp65-CTLs will be further expanded using OKT3, IL2 and autologous feeders as described in our currently active clinical trial using WT1 CTLs (IRB#07-055)

The dendritic cells, EBV BLCL and T-cell lines will be generated in the designated Adoptive Immune Cell Therapy facility of MSKCC under controlled GMP conditions.

Characterization of CMVpp65-specific T-cells

Depending on their growth, T-cell cultures will be evaluated after 21-28 days of culture for the proportion of $CD4^+$ and $CD8^+$ T-cells and content of CD3- $CD56^+$ NK cells and CD20+ Cells. They will also be evaluated for their content of CMVpp65 specific $IFN\gamma^+$ $CD8^+/CD4^+$ T-cells in response to short secondary stimulation with autologous PBMC loaded with CMVpp65 peptide pool. Specificity

will also be ascertained by demonstration of the absence of significant responses to secondary stimulation with unloaded donor and recipient PHA blasts, as well as fully allogeneic EBV BLCL. The matrix of pentadecapeptide subpools approach described by our laboratory (34) will be used to identify specific epitopes inducing IFNγ responses with the $CD8^+$ and $CD4^+$ T-cells. The T-cells will also be tested for their capacity to lyse both CMVpp65 peptide loaded targets and CMV-infected donor and patient PHA blasts. In addition, a panel of single HLA matched EBV BLCL, both loaded and unloaded with CMVpp65, will be used as targets for in-vitro cytotoxicity to determine the HLA restriction of the CMV specific T-cells generated (34, 36).

T-cell cultures providing the required dose of CMVpp65-CTLs and lacking more than background responses to unloaded donor and recipient cells will be cryopreserved for use in adoptive immunotherapy. Immediately prior to cryopreservation, T-cells will be tested by standard techniques for microbiological sterility, absence of mycoplasma, and absence or acceptably low levels of endotoxin (confer appended standard operating procedures). T-cells will be considered acceptable for administration if:

1. They are microbiologuically sterile, free of mycoplasma and contain ≤ 5 EU of endotoxin/kg recipient weight/dose/hour infusion of T-cell culture
2. The T-cells can specifically lyse CMV-peptide loaded donor and /or host APCs.
3. The T-cells do not lyse unmodified PHA blasts of the transplant recipient or, if not evaluable, PHA blasts from a fully allogeneic blood donor above levels of cytotoxicity detected against autologous PHA blasts.
4. Whenever possible, aliquots of these T-cells will be saved to determine the T-cell receptor Vβ repertoire of T-cells reactive against specific epitopes.

Unused T-cells will be maintained cryopreserved under GMP conditions in the Adoptive Immune Cell Therapy facility at MSKCC after the patient's transplant, after which they will be used (i) as 3rd party CMV or EBV specific T-cells if consented to by the donor, for the treatment of other patients such as recipients of cord blood HSCT, or marrow or PBSC HSCT from seronegative donors, or donors who are either not available or did not consent to provide leukocytes for generation of CMV-specific CTLs (ii) for research on viral immunity.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

Patients eligible for this trial will be consenting recipients of related or unrelated stem cell allografts who have an active CMV infection or persistent CMV viremia despite 2 weeks of antiviral therapy or who cannot be maintained on antiviral agents due to therapy related toxicity. If the eligibility criteria are met and a patient is determined to derive benefit from infusion of CMVpp65-CTLs, a standard treatment consent will be obtained and the patient enrolled and treated on this clinical trial.

6.2 Subject Inclusion and Exclusion Criteria

6.2.1 Patient Inclusion Criteria

1. Each patient must satisfy <u>at least one</u> of the following criteria:

a. The patient must have a clinically documented condition associated with CMV (e.g. interstitial pneumonia, hepatitis, retinitis, colitis)

Or

b. The patient must have microbiological evidence of CMV viremia or tissue invasion as attested by viral culture, or detection of levels of CMV DNA in the blood or body fluids consistent with CMV infection.

2. Patient must also satisfy at least one of the following criteria:

a. The patient's CMV infection is clinically progressing or CMV viremia is persistent or increasing (as evidenced by quantitation of CMV DNA in the blood) despite two weeks induction therapy with antiviral drugs.

Or

b. The patient has developed CMV viremia as attested by viral culture, or detection of levels of CMV DNA in blood or body fluids while receiving prophylactic doses of antiviral drugs to prevent CMV infection post transplant.

Or

c. The patient is unable to sustain treatment with antiviral drugs due to drug associated toxicities (e.g. myelosuppression [ANC< 1000µl/ml without GCSF support] or nephrotoxicity [corrected creatinine clearance ≤ 60 ml/min/1.73 m^2 or serum creatinine > 2 mg/dl])

3. Patient has CMV specific T-cells from the donor of his/her HSCT available.

4. CMV infections are life threatening, and may involve multiple organ systems such as the lungs, liver, gastrointestinal tract, hematopoietic and central nervous systems. Antiviral drugs used for treatment may also compromise renal and hematopoietic function. Therefore, dysfunctions of these organs will not affect eligibility for this protocol.

5. Patients must meet the following clinical criteria to receive CMVpp65-CTL infusions
   a. Stable blood pressure and circulation, not requiring pressor support
   b. Evidence of adequate cardiac function as demonstrated by EKG and/or echocardiography.
   c. A life expectancy of at least 3 weeks, even if requiring artificial ventilation.
   d. There are no age restrictions

6.2.2 Patient Exclusion Criteria

1. Patients requiring high doses of glucocorticosteroids (≥ 0.3 mg/kg prednisone or its equivalent)
2. Patients who are moribund
3. Patients with other conditions not related to CMV infection (e.g. uncontrolled bacterial sepsis or invasive fungal infection) which are also life-threatening and which would preclude evaluation of the effects of a T-cell infusion.
4. Patients who are pregnant

6.2.3 Donor Inclusion Criteria

6.1.3a Donors in Group 1 (Historical Donors)

Donors in Group 1 (Section 5.1) would have already been determined to be eligible and will have donated blood or leukocytes to establish CMV-specific T-cells under IRB # 05-065, 07-055, 95-024, or 11-130. There are no additional eligibility requirements for these donors.

6.1.3b Donors in Groups 2 & 3 (Prospective and Volunteer Donors)

Transplant donors and healthy HLA typed volunteers who agree to provide T-cells for Third-party donation (section 5.1, Groups 2 and 3) will need to meet the following eligibility requirements prior to donation:

1. Donors must satisfy the criteria specified in FDA 21 CFR 1271.
2. Donors must be typed for HLA-A, B, C and DR
3. Donors must have a hemoglobin value > 10g/dl
4. Donors must be capable of undergoing, at least, a single standard 2 blood volume leukapheresis or a donation of one unit of whole blood

6.1.4 Donor Exclusion Criteria

1. HTLV/HIV(+) or Hepatitis B or C antigen(+) donors
2. Donors who are known CMV seronegative

7.1 RECRUITMENT PLAN

7.2 Recruitment Procedures

7.2.1 Patient Recruitment

Patients undergoing allogeneic HSCT who are eligible, will be recruited to this trial under the supervision of the allogeneic marrow transplant services in Medicine and Pediatrics.

Prior to receiving treatment, some patients may undergo diagnostic and/or other testing of their tissue, if available, to determine if their CMV infected cells are likely to respond to treatment with CMV specific T cells. Alternatively, blood samples may be required for research tests to ascertain that the CMV-specific T-cells do not contain any cells that could react against the patient. These patients will sign a separate pre-treatment consent.

Eligible patients will be interviewed prior to enrollment on this study, to explain the purpose and the involved treatments and procedures. The risks and benefits of participating in the trial and receiving CMVpp65-CTL infusions will be presented and discussed. These explanatory discussions will include a participating investigator and the patient. A non-participating family member or research nurse should be involved whenever possible.

7.2.2 Donor Recruitment

7.1.2a Donors in Group 1 (Historical Donors)

Because historical donors were only under the care of MSKCC for the period in which they donated cells for their primary recipients and have since returned home, they will be mailed a copy of the consent form to review during the consent discussion, which will occur over the phone. If they agree to 3rd party use of their cells, the consent will be signed and sent back via MSKCC provided pre-paid/addressed envelope.

The registration process is outline in section 15.1.

7.1.2b Donors in Groups 2 & 3 (Prospective and Volunteer Donors)

A similar but separate discussion that occurs with patients will also take place with the prospective donors of the T-cells, defining the risks of blood and/or white cell donations required for the generation of the T-cells to be used for adoptive immunotherapy, and the clinical indications for which the T-cells will be used. Following this discussion, the donor will be asked to sign a written consent to donate the blood cells to be used to generate CMVpp65-CTLs for adoptive immunotherapy.

7.2 Inclusion of Women and Minorities

Memorial Sloan-Kettering Cancer Center has filed form HHS 441 (re: Civil Rights), form HHS 641 (handicapped individuals), and form 639-A (re: Sex Discrimination). In selecting patients for study in the projects proposed in this protocol, we have taken due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations. We expect that the study population will be fully representative of the range of patients seen at Memorial Hospital without exclusion as to age, gender, or ethnic background. Based on a January 1, 2001-December 2005 analysis of patient populations on allogeneic transplant protocols at this Center, the racial distributions were 24% African American, Hispanic or Asian, and 76% Caucasian. The gender distribution was 41% female and 59% male. The protocol is open to all patients irrespective of gender or ethnic background. The population of patients transplanted reflects the distribution of the patients in our tri-state referral area. However, it is anticipated that the distribution of patients by race and ethnicity will be the same for patients receiving marrow or PBSC transplants, but that a higher proportion of individuals from minority racial groups will be accrued to the cord blood transplant protocols, both because of the greater HLA disparity permissible and the concerted efforts of these banks to obtain donor units from populations underrepresented in the NMDP. Given the highly specific patient population that is subject/eligible for this treatment, the trial will be registered under clinical trials.gov to enable other transplant centers to review and refer patients for treatment. No other specific outreach efforts are planned for accrual.

Pregnant women are excluded from this study. There are no age restrictions for this study, and eligible children will be enrolled if appropriate consents are obtained from parents and /or guardians.

8.1 PRETREATMENT EVALUATION

Prior to T cell infusion, eligible and consenting patients will be clinically assessed for their general condition and for extent of CMV infection. Tests/exams will be obtained within 3 days of infusion (whenever possible). Certain tests may be omitted at the discretion of the treating physician:

- Clinical history and Physical examination
- Chest x-ray (not necessary if CT scan is done)
- CT scan is not required prior to treatment, but should be done, whenever possible, within 3 days of T cell infusion
- Ophthalmologic exam for CMV retinitis, if clinically indicated
- Colonoscopy for CMV colitis, if clinically indicated
- ECHO and/or EKG, if clinically indicated
- CBC with differential
- Comprehensive Panel
- Cultures of affected body fluids or tissues will be obtained whenever possible
- CMV viremia will be assessed by quantitation of CMV antigen+ blood leukocytes and by quantitation of CMV DNA in the blood by realtime quantitation PCR.
- Lymphocyte immunophenotype (BMT short panel) and T cell responses to PHA and Candida Albicans

In addition, T cell responses to CMV will be evaluated in Dr. O'Reilly's laboratory by:

- Assessment of T cell proliferation in response to CMV antigens and
- Quantitation of T cells generating IFNγ in response to stimulation with a pool of CMV pentadecapeptides and
- In patients expressing HLA-A0201, B0701 or B0801 T cells binding CMV peptide – HLA A2, B7 or A24 tetramers
- Analysis of TCR Vβ repertoire will also be performed

9.1 TREATMENT/INTERVENTION PLAN

This is a single institution phase II trial designed to assess the therapeutic activity of primary donor derived CMVpp65 specific T-cells (CMVpp65-CTL) for the treatment of CMV infection or persistent CMV viremia after allogeneic hematopoietic stem cell transplantation. The T-cells will be generated from healthy, CMV seropositive donors by sensitization *in vitro* with autologous, cytokine-activated monocytes loaded with a pool of overlapping synthetic peptides spanning the sequence of CMV protein pp65.

9.1 Treatment with CMVpp65 Specific T-cells

Eligible patients who consent to enter this trial and for whom CMV-pp65 CTLs are available, will receive 3 weekly infusions of CMV-pp65 CTLs. The T cells will be administered immediately after thawing. The final product is cryopreserved in a volume of 1-2 ml. This will be drawn up in a sterile syringe containing 30 ml of normosol for intravenous administration and administered by slow intravenous infusion ideally over approximately 5 minutes.

In this phase II trial, patients will be treated at doses of 1 x 10^6 CMVpp65-CTL/kg/dose/week for 3 weeks (+/- 20% variability of total dose). Patients will be observed for the following 3 weeks. Additional 3 week courses of CMVpp65-CTL may be administered if levels of CMV DNA in blood are still detectable despite disease stabilization or improvement.

If the planned dose of CMV peptide sensitized T-cells is not achieved, but the cells generated exhibit required levels of CMV specific cytotoxic activity and meet all other release criteria, the T-cell product may be administered at a lower dose level.

In certain cases, in order to avoid refreezing of the final product to achieve a specific dose level, which may affect viability, variability of each dose may exceed +/-20%. In such cases, care will be given to ensure that the total combined volume of the 3 doses does not exceed the planned dose.

9.2 Subject Stratification

Subjects will include patients treated with CMVpp65-CTLs derived from their transplant donor. These will be patients with CMV seropositive transplant donors who have previously provided leucocytes for generation of CMVpp65-CTL and for whom such CMVpp65-CTL are available.

9.3 Selection of CMVpp65-CTL

Patients will be treated with CMVpp65-CTL generated from their transplant donor.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

The timing of evaluations below is approximate. The schedule of tests described should be adhered to whenever possible. However, in certain situations, particularly late after treatment, circumstances may preclude assessments at the times specified, at which point they may be held the discretion of the treating physician:

- Patients will be evaluated clinically by vital signs pre-infusion, post, and at approximately 1, 2, 3, and 4 following initiation of each infusion of CMV-specific T-cells

- The patient should be followed weekly for the first 8 weeks post-initial infusion with CMV-specific T-cells, then monthly until 6 months post initial infusion. Patients will receive interim history, review of systems, and physical exam (including GvHD evaluations) on the day of infusions, and on days 1, 8 and 15 whenever possible.

- Patients should be monitored by CBC and comprehensive metabolic panels weekly for the first 8 weeks post-initial infusion with CMV-specific T-cells, then approximately monthly until 6 months post initial infusion.

- Tests quantitating CMV viremia by CMV DNA copies per milliliter of blood should be obtained weekly for the first 8 weeks post-initial infusion with CMV-specific T-cells, then approximately monthly until 6 months post initial infusion

- Quantifications of T cells generating IFNγ in response to pooled CMV pentadecapeptides or T cells specific for single pentadecapeptides known to be contained in the T cells infused should be measured weekly for the first 8 weeks post-initial infusion with CMV-specific T-cells, then monthly until 6 months post initial infusion. If the T cells infused are known to contain T cells that can bind specific CMV peptide/HLA tetramers, these T cells will also be quantitated.

- When possible, pre-initial infusion and at week 4 and month 6 post initial infusion, if >1% of the circulating T cells are found to be reactive against specific epitopes by either assay, these CMV-pp65-reactive T cells will, if possible, be characterized as to their T cell receptor Vβ repertoires, to determine whether and to what degree, the T cells expanding *in vivo* resemble (and, therefore, are likely to be derived from) the CMV-specific T cells infused at the time of adoptive transfer

- If there are clinical indications, research samples may be collected testing for cytokines.

- Flow Cytometry 7 panel to enable quantification of CD4 and CD8 T cell number may be obtained prior to the first infusion, on day 14, week 4, and week 8 post-initial infusion with CMV-specific T-cells, then monthly until 6 months post initial infusion

- T cell populations and their responses to mitogens and to candida albicans antigen should be obtained pre-initial infusion and may also be monitored at approximately 2, 4, and 6 months post initial infusion as a measure of general immune function.

- Patients with CMV pneumonia should be monitored by at least daily measurements of blood gases and/or O2 saturations until normalized and weekly chest films together with CT scans at pre-infusion and approximately every 2-4 weeks until clear.

- Ophthalmologic evaluations will be made to document responses of CMV retinitis. These evaluations will ideally be done every 2 weeks.

- Patients with known CMV enteritis should be monitored by approximately three times weekly measurements of 24 hour stool volumes, stool guaiacs and cultures for CMV. If acceptable to the patient, colonoscopy should be repeated approximately three weeks after the dose of T cells to assess response.

- If the CMV infection affects the CNS, lung, or abdominal organs, and virus has been detected (e.g.: in pleural or peritoneal fluids), appropriate tissue and/or CSF may be collected (if indicated and possible) and tested for the presence of CMV and CMV specific T-cells. These tests should occur between 3-6 weeks post initiation of treatment, whenever possible.

APPROXIMATE SCHEDULE OF PATIENT EXAMINATIONS AND STUDIES

Evaluations	Pre-Infusion	Day 0 -> 1	Day 7	Day 14	Day 15	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Mo 3	Mo 4	Mo 5	Mo 6
History, Review of Systems, & Physical Exam (incl. GVHD eval)[1]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CBC/CMP	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CMV PCR (10 ccs)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
T cell Immunophenotype short panel	X			X			X				X	x	x	x	x
Mitogens (PHA)	X										X		X		X
Antigen (Candida albicans)	X										X		X		X
Research Bloods	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immune Monitoring[3]	X		X	X		X	X	X	X	X	X	X	X	X	X
Chest X-Ray and/or CT (if indicated)	x														
EKG (if indicated)	x														
Ophthalmologic exam (if indicated)	x														
Colonoscopy (if indicated)	x														
Cultures of affected body fluids and/or tissue (if possible/indicated)	x					x									
Treatment CMV Specific T-cells[2]		X	X	X											

[1] GVHD evaluations to be performed weekly until Week 8 and a consensus at Day 100
[2] If patient is given additional cycles of CMV specific T-cells, calendar will restart from Day 0. Doses are given weekly and can be held at the discretion of the PI or in the case of severe toxicity related to infusion.
[3] Prior to and at specific intervals following infusion of CMVpp65-CTL, patients will be monitored for levels of CMVpp65-CTL in the blood as measured by quantitation of CTL precursor frequencies, CMV pp65-specific IFNγ+ T-cells (35), or epitope specific-T-cells binding HLA-peptide tetramers and their Vβ usage (36). The third party T-cells will also be monitored by analysis of CMVpp65 IFNγ+ T-cells as to their genetic origin.

11.0 TOXICITIES/SIDE EFFECTS

In this study, we will be capturing and tracking Grade 3-5 toxicities which occur within 30 days following an infusion of CMV infusion CTLs and are potentially attributable to treatment on study. We will also be capturing and tracking graft vs host disease occurring at any time point post-infusion. Patients who might subsequently receive donor lymphocytes, boosts, or a secondary transplant will be evaluable up until that time point. Toxicities which are attributable to underlying malignancy and/or were present prior to initiation of therapy will not be tracked. Please see section 17.2 for Serious Adverse Event reporting.

1. **Acute Toxicities and Transfusion Reactions.** The infusion of CMV-peptide specific T-cells might cause an acute transfusion reaction or an allergic reaction to the cell infusion manifested by any one or all of the following clinical signs and symptoms: fever, shaking chills, difficulty breathing, a drop in the blood pressure, skin rash or swelling of the mucus membranes in the mouth or vaginal tract, or impairment of kidney function. Based on prior experience with infusions of T-cells at our own and other

institutions, the risk of such immediate toxicities is anticipated to be small. However, each patient will be closely monitored for vital signs and clinical symptoms prior to and following T-cell infusion. Reactions will be treated as clinically indicated.

2. **Specific Immune Reactions.** The CMV peptide-specific T-cells are expected to migrate to sites where CMV infected cells are concentrated, and to begin to kill these CMV infected cell populations. These cells may be concentrated in the lung, liver, retina/central nervous system, or intestinal tract. While it is expected that the small doses of T-cells administered will take time to replicate, and will exert their antiviral effects over a time period of days to weeks, it is possible that these cells could concentrate in an infected tissue and cause more severe inflammation as they attack virus infected cells. This could transiently increase the severity of a pneumonia or a CMV –induced encephalitis, retinitis, hepatitis or intestinal infection, and could even be life-threatening. Additionally, patients with pre-existing pulmonary conditions maybe at increased risk for development of pulmonary toxicity including diffuse alveolar hemorrhage. For patients treated for drug resistant CMV-retinitis, these T cells could potentially cause partial or complete blindness. If this were the case, immunosuppressive drugs might be required to halt such a reaction, and eliminate the T-cells. Were this required, the antiviral activity of the T-cells would be removed and the infection could progress in the absence of any specific resistance.

3. **Graft versus Host Disease.** The CMV peptide-specific T-cells have been cultured for over 4 weeks. During this time, T-cells reactive against other antigens, including alloantigens that might be expressed by tissues in the patient, die off and are deleted. The T-cells to be infused will be tested and shown to have no significant activity against normal allogenic cells prior to their infusion. However, there is a finite risk that small numbers of alloreactive T-cells could be transferred in the T-cell inoculum, which could cause Graft versus Host Disease (GvHD).This immune reaction against the host can cause skin rash, hepatitis, and enteritis. In its most severe form, it can be fatal. Were moderate to severe GvHD (Grade 2-4) to develop, the patient will be treated with high doses of glucocorticosteroids. Additional immunosuppressive agents may be used if the GvHD does not respond to steroid treatment.

4. **Infection.** There is a potential that transfusions of white cells or T-lymphocytes may also serve as vectors of serious infection. All precautions to maintain sterility will be taken.

5. **Autoimmune Hemolytic Anemia**. Autoimmune hemolytic anemia has been reported in 2 out of 75 (2.6%) patients treated with CMV peptide-specific T-cells in prior phase 1 and phase 2 clinical study experience. Both SAEs were grade 3 events., and one event was considered possibly related to CMV peptide-specific T-cells while the other was considered to be unrelated. Patients who develop autoimmune hemolytic anemia should be managed per institutional standard. Discussion with the medical monitor is encouraged.

11.1 Donor Toxicity/Side Effects

Risks of blood donation are minimal and include: minor discomfort at site of blood draw, some bleeding and/or bruising, and lightheadedness.
Risks of Leukapheresis include: The agent used to prevent clotting of blood during a leukapheresis may cause low levels of calcium in the blood and result in the lack of feeling or tingling of fingertips or around mouth, which normally last only a short time, and can, potentially, progress to cramps. This is normally treated with a calcium supplement.

Other leukapheresis side effects include:
- Pain

- Bruising at the needle insertion sites
- Dizziness
- Nausea
- Fainting due to temporary lowering of the blood pressure (rare)
- Infection (rare)

Platelet count may drop during the leukapheresis and will be measured after the collection. If platelet count falls enough to place the donor in danger of bleeding, count will be checked daily until it returns to normal.

The leukapheresis requires that a needle be placed in a vein in each arm. About 15% of the time, we are unable to use the donor's arm veins. The donor may be asked to go through a minor surgical procedure to place a central line. The donor will be asked to sign another consent form if a central line needs to be put in.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary endpoint of this study is to assess whether the infusion of the CMVpp65-CTLs has therapeutic activity in curing CMV infections in recipients of HSCT. This study specifically will evaluate the therapeutic efficacy and persistence after infusion of CMVpp65-CTLs derived from the HSCT transplant donor for the treatment of CMV infections. The second clinical end point of this study is the evaluation of GvHD induction after infusion of donor derived CMVpp65-CTLs. .

The responses of CMV infections will be clinically monitored as detailed under section 10. Responses to adoptively transferred T-cells are usually not observed until the T-cells have had time to expand within the transplant recipient, a period of at least 3 days, and as many as 18-21 days after the T-cell infusion. Accordingly, initial patient responses will be scored at weekly intervals, from day 7-day 21 post the initial infusion. Final responses will be captured in CRDB and scored between days 28-42 post the final infusion. Outcome responses will be captured post-first cycle and post final-cycle of infusions of CMV specific T cells. Best response observed in the full course of treatment will also be recorded as a distinct entity.

Clinical responses will be considered complete if all manifestations of CMV disease have resolved and/or the patient no longer has detectable CMV DNA by PCR.

A partial response will be defined as a $2xLog_{10}$ reduction in the level of CMV DNA detected in the patients blood and/or improvement in clinical parameters of CMV infection such that the patient is symptom-free.

Stable disease will be defined as no change in the clinic severity of disease in any organ.

Progressive disease will be defined as disease progression by clinical and radiologic parameters, ascribable to CMV infection in any affected organ, with unchanged or increased levels of CMV DNA.

Graft versus Host Disease initially developing after 10 days post T-cell infusion will be considered to be potentially ascribable to the T-cell infusion. Graft versus Host Disease will be diagnosed and clinically graded using the standard criteria of the IBMTR/NMDP Consensus Panel. Whenever, possible, the diagnosis will be confirmed by biopsy of skin or other involved organ, using the pathological criteria of Woodruff et al. Patients with preexisting GvHD of grade 1 severity not requiring

systemic steroids, or patients with a prior history of GvHD who have resolved this process sufficiently so that they no longer require parenteral steroid treatment will be considered to have developed an exacerbation or worsening of GvHD ascribable to the T-cell infusion if they redevelop clinical evidence of grade 2 or greater GvHD 7 or more days after a dose of T-cells.

For the evaluation of toxicities, the NCI Standard Toxicity Scale 4.0 will be employed.

13.0 CRITERIA FOR REMOVAL FROM STUDY

In accordance with the Declaration of Helsinki, ICH Good Clinical Practice Guidelines, and the US FDA Regulations, a patient has the right to withdraw from the study at any time for any reason without prejudice to his/her future medical care by the physician or at the institution. The Investigator also has the right to withdraw patients from the study (see below). Should a patient (or a patient's legally authorized representative) decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

A complete final evaluation should be made at the time of the patient's withdrawal, the Study Status Outcome form in the case report form should be completed with an explanation of why the patient is withdrawing, and an attempt should be made to perform a follow-up evaluation.

Patients may be removed from study if one or more of the following events occur:

- Significant noncompliance on the part of the patient
- Refusal of the patient to continue treatment or observations
- Unacceptable toxicity (i.e.: grade 3-4 toxicity related to CMV CTL infusion). Removal for unacceptable toxicity does not necessarily preclude continued follow-up.
- Progressive disease that in the Investigator's opinion requires therapeutic intervention that would interfere with the interpretation of results from the study
- Decision by the Investigator that termination is in the patient's best medical interest
- Unrelated medical illness or complication
- Lost to follow-up.

14.0 BIOSTATISTICS

A phase 2 trial design will be applied to determine the efficacy and safety of CMV specific T cells for the treatment of marrow transplant patients with CMV. The endpoint of this study is complete response, defined as the clearance of the CMV infection 3-7 weeks following completion of the last cycle of CMV CTLs. The evaluation of treatment efficacy will be assessed for patients receiving CMV specific T cells from their transplant donor. It is anticipated that accrual will be completed within 3 years.

A two-stage design with a maximum of 39 patients will be employed. The study design discriminates between population response rates of 0.50 and 0.70. In the first stage, 23 patients will be enrolled in the study. If 11 or fewer complete responses (CRs) are observed, then the trial will be stopped. If at least 12 complete responses are observed, then 16 additional patients will be accrued. At the conclusion of the trial, if 23 or fewer CRs are observed, we will conclude that the treatment is not sufficiently active. This design has power 0.90 for a population CR rate equal to 0.70 using a one-sided test with type 1 error equal to 0.10.

The probability of a complete response and the proportion of patients requiring ganciclovir or foscarnet will be computed.

Following approval of Amendment 22, additional accrual on study is anticipated to be 2-3 patients per year, for an additional 3 years, for a target accrual of approximately 7 additional patients. As the accrual will be lower than the originally targeted accrual number, there will be no hypothesis test for efficacy analysis and the efficacy analysis is to estimate the complete response (CR) rate with its 80% confidence interval. For example, with 9 CR observed among a total of 15 subjects, the estimate of CR rate is 60% with the 80% Clopper-Pearson exact confidence interval of (40.4%, 77.4%).

The effects of infusing activated CMV-specific T-cells on the general immune function of the host will be assessed by recording $CD4^+$ and $CD8^+$, $CD3^+$ T-cells, NK cells and B-cells prior to and one week following each course of cell infusions and 1, 2 and 4 months following completion of the second course of cells. In addition, the levels of CMV DNA and T cells generating IFNg in response to the CMV pentadecapeptides will be measured weekly for the first 8 weeks and then monthly until 6 months post infusion. Nonparametric smoothing will be used to estimate the population trajectory of these response curves over time. In addition, a generalized estimating equation approach will be used to model the relationship between the immunologic reconstitution factors and complete response.

In order to reduce patient risk, the study design includes early termination in the event of excessive graft versus host disease during the accrual period. The stopping rule for excessive GvHD and the corresponding power calculation will be applied separately for the two donor groups. In the event that the stopping boundary is crossed for one of these groups, the study will continue accrual in the other group.

Allo donor

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Acute GvHD (grades 3-4)	3 within the first 15 patients	0.06	0.10
	4 within the first 24 patients 5 within the first 35 patients 6 within 39 patients	0.20	0.90
Chronic GvHD (extensive)	3 within the first 15 patients	0.06	0.10
	4 within the first 24 patients 5 within the first 35 patients 6 within 39 patients	0.20	0.90

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.3 Randomization

There is no randomization in this study.

16.1 DATA MANAGEMENT ISSUES

This is a single institution trial and all patients will be treated at Memorial Sloan-Kettering Cancer Center.

A Clinical Research Coordinator (CRC) will be assigned to this study. The responsibilities of the CRC include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data manager will also monitor laboratory compliance through out the study. Laboratory data will be tabulated and summarized based on MSKCC normal ranges.

The data collected for this study will be entered into the MSKCC Clinical Research Data Base (CRDB).

16.2 Quality Assurance

Registration reports will be generated by the CRC on a regular basis to monitor patient accruals and completeness of the registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring Plan utilized for this study must align with the MSK DSM Plan, where applicable.

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan Kettering were approved by the National Cancer Institute in August 2018. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials.”

There are several different mechanisms by which clinical studies are monitored for data, safety and quality. At a departmental/PI level there exists procedures for quality control by the research team(s). Institutional processes in place for quality assurance include protocol monitoring,

compliance and data verification audits, staff education on clinical research QA and two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: Data and Safety Monitoring Committee (DSMC) for Phase I and II clinical trials, and the Data and Safety Monitoring Board (DSMB) for Phase III clinical trials, report to the Deputy Physician-in-Chief, Clinical Research.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required.

The MSK DSMB monitors phase III trials and the DSMC monitors non-phase III trials. The DSMB/C have oversight over the following trials:

- MSK Investigator Initiated Trials (IITs; MSK as sponsor)
- External studies where MSK is the data coordinating center
- Low risk studies identified as requiring DSMB/C review

The DSMC will initiate review following the enrollment of the first participant/or by the end of the year one if no accruals and will continue for the study lifecycle until there are no participants under active therapy and the protocol has closed to accrual. The DSMB will initiate review once the protocol is open to accrual.

17.1 PROTECTION OF HUMAN SUBJECTS

17.2 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with others at the time of study publication.

17.3 Serious Adverse Event (SAE) Reporting

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the participant's condition
    - Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

17.2.1

17.2.2 Definition of SAE

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

Attribution:

- Unrelated: The AE *is clearly NOT related* to the intervention
- Unlikely: The AE *is doubtfully related* to the intervention
- Possible: The AE *may be related* to the intervention
- Probable: The AE *is likely related* to the intervention
- Definite: The AE *is clearly related* to the intervention

Expected and Unexpected Event:

- Expected: Any experience *previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan
- Unexpected: Any experience *not previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

Participants enrolled to the "historical donor" arm are, frequently, no longer under the care of MSKCC. This is because the primary patients, for whom their donations were initially intended, have completed treatment and are no longer at risk for CMV. Donors who fall into this category will be mailed a copy of the consent form to review during the consent

discussion, which will occur over the phone. If they agree to 3rd party use of their cells, the consent will be signed and sent back via MSKCC provided pre-paid/addressed envelope.

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