MSK PROTOCOL COVER SHEET

A Phase II Trial of Adimlecleucel (Third Party Donor Derived CMVpp65 specific T-cells) for The Treatment of CMV Infection or Persistent CMV Viremia after Allogeneic Hematopoietic Stem Cell Transplantation, solid organ transplant, human immunodeficiency virus, other immunocompromised states, and immune competent subjects who require therapy

Principal Investigator/Department:Kevin Curran, M.D./Pediatrics

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 HLA partially matched third party donor-derived CMVpp65 specific T-cells (adimlecleucel) for the treatment of CMV infection or persistent CMV viremia after allogeneic hematopoietic stem cell transplantation solid organ transplant, human immunodeficiency virus, other immunocompromised states, and immune competent subjects who require therapy. T-cells for infusion will be generated from seropositive healthy third party donors, 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 third party donor derived adimlecleucel 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 by:
    a. Partial or Complete decrease in CMV DNA viral load
    b. Partial or Complete resolution of CMV disease
2. Determine the risk of inducing GvHD or increasing its severity by adoptive transfer of third party donor derived adimlecleucel.

Secondary Objectives:

1. Determine safety data regarding the use of third party donor derived adimlecleucel for the treatment of CMV viremia and disease.
2. Determine the degree to which treatment with adimlecleucel can eliminate or reduce the exposure to antiviral agents such as ganciclovir and foscarnet and thus reduce the associated morbidity from organ specific toxicity of antiviral agents.

3.0 BACKGROUND AND RATIONALE

Cytomegalovirus (CMV) is a member of the herpes virus family of deoxyribonucleic acid (DNA) viruses, which can infect several types of cells within the body (epithelial, endothelial, hematopoietic, and connective tissue cells). CMV is a large virus encoding approximately 200 proteins that, like other herpes viruses, remains latent in the host permanently and may reactivate at times of immunosuppression or compromise, though the cellular site of latency is not well understood. T cell-mediated immunity is the primary immunological control for CMV and loss of this immunity can result in reactivation of CMV into the lytic phase and progression to organ involvement with consequent injury or death (1).

Clinically significant CMV reactivations follow allogeneic hematopoietic cell transplant (alloHCT) and solid organ transplant (SOT). Despite prophylactic and pre-emptive therapy, reactivation of latent

CMV infections remain a cause of morbidity and mortality in these populations. In alloHCT patients, reactivation most commonly occurs between 30 to 100 days after transplant, with a median of about 40 days after transplant (2).

Reactivation rates vary among seropositive HCT recipients based on the conditioning regimen used, patient age, and source of stem cells, with T cell depletion and cord-blood transplants resulting in the highest reactivation rates. It is noteworthy, however, that T-cells depleted grafts from seropositive donors do not transmit CMV to seronegative recipients. The increasing use of haploidentical alloHCT appears to result in increased risk of reactivation (3, 4). Although prophylactic or preemptive treatment with ganciclovir or foscarnet has reduced the incidence and mortality of early CMV infections in HLA-matched recipients(5, 6), in 6-30% of cases, treatment may be hampered or cannot be sustained due to complicating myelosuppression or nephrotoxicity (7, 8). 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 (9, 10). These late infections were lethal in 46% of cases, and were associated with persistent deficiencies in absolute numbers of $CD4^+$ and $CD8^+$ T-cells and CMV-specific T-cells (9, 10). In our own study of 255 recipients of HLA-matched $CD34^+E^-$ T-cell depleted HCT who did not receive post transplant immunosuppression, the cumulative incidence of CMV reactivation among seropositive patients was similar to that reported following unmodified HCT (58% vs 40-60%), and only 12% developed clinically overt disease (11). However, similar to findings reported for unmodified allo HCTs, deficiencies of $CD4^+$ and $CD8^+$ T-cells were significantly associated with disease (10, 11).

CMV infections are particularly dangerous in seropositive recipients of HLA non-identical HCT. For example, while the overall mortality of CMV infections in recipients of HLA-matched HCT has been reduced to 3-5% (12, 13), mortality rates of 13-16% have been reported among recipients of HLA-haplotype disparate T-cell depleted grafts (14). 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 (15-17).

SOT patients may receive prophylactic therapy prior to CMV reactivation or pre-emptive therapy at the time of CMV viremia detection to reduce the risk of progressive CMV infection. Approximately 1% of SOT patients develop ganciclovir resistance (18), which can lead to organ damage, organ loss, and sometimes death (19). Patients undergoing lung transplant may have higher rates of ganciclovir resistance, ranging from 3%-10% (20, 21).

In SOT patients, CMV infections are most common in donor seropositive/recipient seronegative transplants since the recipient does not have endogenous CMV immunity and will be chronically immunosuppressed to reduce risk of allograft rejection. In the alloHCT setting, donor seronegative/recipient seropositive transplants are the highest risk since the donor does not transfer endogenous CMV immunity to the recipient at the time of transplant and development of CMV directed immunity requires immune reconstitution.

<u>Adoptive Immunotherapy for CMV</u>

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 (22). 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 (23-25). 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 HCT (26-38). Our group provided the first demonstration of the potential of DLI to treat EBV lymphomas in 1994 (33). 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 HCT. In our initial experience, three weekly infusions of 10^6 EBVCTL/Kg recipient weight induced durable CRs in 13/19 (68%) patients treated (30, 31).

Riddell et al.(39) 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 HCT treated for persistent CMV viremia unresponsive to antiviral drugs with infusion of 5 x 10^5 – 1 x 10^6 CMV specific T-cells/kg or 10^3- 10^4 CMVpp65 responsive IFNγ$^+$ T-cells/kg (29, 35-37). Thus studies by Peggs et al (29), and Einsele et al (35), 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 HCT and demonstrated successful clearance of CMV viremia and reconstitution of CMV specific immune responses in 19/24 treated patients, with no grade 2 or greater GvHD. Micklethwaite et al.(36) 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 HCT with only 4/12 patients developing transient CMV viremia and no increased incidence of GvHD in this prophylaxis trial. Feuchtinger et al.(37) treated 18 patients after allo-SCT from HLA–mismatched/ haploidentical or HLA–matched unrelated donors with 2 x 10^4 IFNγ$^+$ 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 (29, 35), 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) (37).

Our phase I trial of transplant donor-derived CMVpp65 peptide specific T-cells (IRB 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 6 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 CMVpp65CTLs were sustained for 2-4 months. Acute toxicities were rare, no patient developed de novo GvHD.

Despite the promising results of these trials of adoptive immunotherapy with CMV-specific T-cells, there are logistic, immunologic and immunogenetic constraints to the broad application and/or effectiveness of adoptive T-cell therapies that remain formidable and, until recently, have been intractable. First among these is the difficulty of treating CMV infections in seropositive recipients of transplants from seronegative, immunologically naïve donors, specifically, previously unexposed adults or, as is now most common, cord blood transplants (CBT). For most of these cases, CMV-specific T-cells cannot be generated from the transplant donors ex vivo. While virus -specific T-cells can sometimes be generated from CBT (40), the donor-derived cells required to generate the T-cells are often no longer available. Furthermore, the T-cells generated are of low affinity and as yet minimally tested in terms of therapeutic efficacy (40).

The second major limitation is logistic in nature; the acuity and severity of CMV infections demand prompt therapy, but 4-6 weeks of in vitro culture are required to generate CMV-specific T-cells of appropriate specificity that are adequately depleted of contaminating alloreactive cells capable of causing GvHD. CMV-specific T-cells can be isolated directly from donor-derived leukapheresis by isolation of CMV peptide/HLA tetramer+ T-cells of after short-term sensitization with antigen-presenting cells expressing CMV proteins or loaded with immunogenic CMV peptide by immunoadsorption of T-cells expressing activation markers or secreting interferon γ. However, the yields of T-cells isolated in this way are limited and their potential to clear clinically overt infection is thus far largely untested (29, 35). Furthermore, such preparations still incur a risk of GvHD even in HLA-matched recipients.

The third limitation affects patients who have received transplants from HLA disparate donors, and derives from the fact that T-cells isolated directly from the blood or generated in vitro after sensitization with CMV lysates or peptide pools are usually responsive to only 1-3 CMV immunodominant peptide epitopes presented by one or two alleles of the donor. As a result, such T-cells are ineffective in an HLA disparate transplant recipient if they are restricted by HLA alleles not shared by the patient, since they cannot recognize or kill patient cells infected with the virus.

A promising new approach that addresses these limitations and can be rapidly applied to the treatment of life threatening CMV and EBV infections. Haque et al (32) provided initial demonstrations that 3rd party EBV-specific T-cells selected only on the basis of partial HLA matching, could induce complete remissions (CR) of EBV-LPD complicating organ allografts. In their initial study of 8 patients, 3 achieved CR (41). In a subsequent multicenter study of 33 patients 13 (39%) achieved sustained CRs (32). Subsequently, we demonstrated that adoptive transfer of HLA partially matched third party donor-derived EBV-specific T-cells could also induce durable CRs in two patients who developed Rituximab-resistant monoclonal EBV lymphomas following allogeneic cord blood transplants (22). We have now used third party EBV CTL to treat 46 patients with Rituximab and/or chemotherapy resistant EBV lymphomas that developed after allogenic HCT (N=33), or organ transplants (N=13), (Protocol 95-024 and 11-130, IND 6834) (23). Of the initial 33 HCT recipients, 22 achieved durable CRs (N=19) or PRs (N=3); of the 13 solid organ recipients, 3 achieved sustained CRs and 6 PRs lasting over 1.5 years post treatment. The durability of these responses has been striking particularly since the third party T-cells engraft only transiently and are rarely detected in the blood for more than 28 days. In these patients, no patient has developed graft rejection and one patient developed grade I skin graft versus host disease that responded to topical therapy.

In the course of our Phase I trial evaluating transplant donor-derived CMVpp65-specific T-cells for adoptive therapy of patients with overt CMV disease or CMV viremia persisting for more than 2 weeks despite antiviral therapy (IRB 05-065; IND 13244) we introduced an amendment to also permit the use of HLA partially matched CMVpp65 –specific T-cells from third party donors for patients whose own donors were either seronegative (e.g. cord blood or seronegative adult donors) or were unavailable or unwilling to provide cells for adoptive therapy.

151 GMP grade CMVpp65-CTL lines that have been fully characterized as to their CMVpp65 specific cytotoxic activity, epitope specificity, and HLA restriction. When the protocol was amended to permit use of third party donor-derived CMVpp65 specific T cells, we also obtained a separate consent from each of the donors to use a portion of their cells for adoptive therapy of patients other than the patient to whom they donated a HCT. In that amended study, of 10 evaluable patients who received third party CMVpp65-specific T-cells after failing treatment with at least 2 antiviral drugs, 3 achieved a complete response, 2 a partial response with at least a reduction in CMV as measured by antigenemia or viremia by PCR, and 1 had stable disease. Two patients continued to progress and died of infection. Two received only one infusion and were evaluable for toxicity only, although one of these patients cleared CMV.

In 2012, we opened a two armed phase II trial to compare the antiviral activity of transplant donor-derived CMVpp65-specific T-cells versus CMVpp65 specific T-cells from HLA partially matched healthy HCT donors other than the donor of the HCT administered to the affected patient (i.e. third-party donors), in the treatment of CMV infection or persistent viremia. This protocol (IRB 12-086A(6)) was approved by the MSKCC and FDA. Thus far, 23 patients have accrued to this study for treatment with third party donor-derived CMVpp65 specific T-cells, of whom 20 have been followed long enough to be evaluable. Of these 20, 5 achieved a CR and 7 a PR, and 3 stable disease. Only 5 patients have had progressive disease. Furthermore, 3 patients treated with CMV-CTLs have developed denovo GvHD or a flare of existing GvHD during the GvHD evaluation period. Of these 3, only 1 was considered possibly related to CMVpp65-specific T-cells.

Subsequently, the FDA has requested that the phase II study of third party donor-derived CMV T-cells be conducted on a distinct protocol. In a response to this request, we opened this single-armed Phase II trial to evaluate third party derived CMVpp65CTL for treatment of CMV infections of HCT recipients of bone marrow or PBSC HCT from whom CMV-specific CTLs from their transplant donor were not available. (IRB 14-070).

Thus far, we treated 50 transplant recipients with third party donor derived CMVpp65-specific T-cells between 10/14/11 and 11/28/16, evaluable for response assessment as of 6/20/17. Patients had received an unmodified (N=11) T-cell depleted HCT (N=33) or cord blood (n=6), transplant. Fifteen were treated for overt CMV disease involving CNS (N=6), GI (N=10) and Lung (N=2) and 35 for CMV viremia persisting despite >2 weeks of induction therapy with 1-3 antiviral agents. Patients had received a median of 3 (1-6) prior antiviral treatments.

Third party CMVpp65-CTLs were selected from a bank of lines generated under GMP conditions from normal HCT donors who specifically consented to use of their T cells in patients other than their designated transplant recipient. Selection was made on the basis of HLA restriction by at least one HLA allele shared by the patient and HCT donor, and matching for $\geq$ 2/10 recipient alleles. If such a line was not available, a patient could be treated with a line matched at only one HLA allele as long

as the restriction was through that matched allele. Patients received 3 weekly infusions of approximately 1x106 CMVpp65-CTL/kg/infusion. Patients were sequentially evaluated for clinical and radiographic changes, quantifications of CMV DNA by PCR and IFN+ CMVpp65-specific T-cells in the blood. Responses were assessed 28-42 days after the first of each cycle of CMVpp65-CTLs. Response in patients with CMV disease was considered complete (CR) if all sites were cleared of virus by biopsy and blood sampling and partial (PR) if symptoms resolved and viremia met criteria of PR. In patients treated for persistent viremia, responses were complete if CMV DNA was cleared in repeated testing, and partial if the level of CMV fell based on the testing method by >50% (N=2) or by 2xLog10 (N=12).

Of the 50 patients 18 had a complete and 14 a partial response for an overall response rate of 64%. Response rates in patients with disease (5CR+4PR/15) were similar to those of patients with persistent viremia (13CR+10PR/35). In patients treated for viremia alone, survival at 6 months was 65.7% and in those with disease 60.0% (a). More extensively pretreated patients who received CMVpp65 CTLs > 100 days post CMV initial detection fared as well as those treated earlier (62.1% vs. 66.7% OS) (b). Patients who responded to CMVpp65-CTL therapy (CR or PR) had an improved survival with 6 month overall survival of 81.3% (b) and 12 month overall survival of 62.1% (c); only 1 of these 32 patients died of CMV. In contrast 7 of 18 non-responding patients died of CMV; overall survival in this cohort was 33.3% at 6 months. By 12 months, 8 non-responding patients had died of CMV and overall survival had decreased to 22.2%.

Toxicities associated with CMVpp65-CTL infusions in this cohort are limited with 5 patients experiencing adverse events of > grade 3 severity deemed possibly related to CMVpp65-CTL therapy. Two of these patients died, one due to sepsis and one due to progression of CMV.

This study demonstrates a high response rate among patients with otherwise refractory CMV viremia and disease. The bank of CMVpp65-CTLs can provide an immediate source of HLA partially-matched appropriately restricted T cells for adoptive immunotherapy to treat persistent CMV viremia and CMV disease, including disease isolated to the CNS. The availability of 3rd party CMVpp65-CTLs enables treatment early in the course of disease and may thereby improve response rates while minimizing toxicity from anti-viral therapy.

In addition to these on protocol patients, we have treated patients on single patient use/eINDs for refractory CMV occurring after SOT (N=2) and HIV (N=2). These patients have not experience a toxicity profile to suggest different toxicities than HCT patients who have received CMVpp65-CTLs and no serious adverse events were submitted.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a single-arm non-randomized single institution phase 2 trial, designed to evaluate the therapeutic activity of adimlecleucel generated from healthy seropositive third party donors when adoptively transferred into allogeneic hematopoietic cell transplant recipients, solid organ transplant (SOT) recipients, human immunodeficiency virus, other immunocompromised states, and immune competent subjects with persistent CMV infection or viremia.

Individuals eligible for this trial will be patients who have an active CMV infection or persistent CMV viremia despite treatment with anti-viral agents for ≥ 2 weeks or who cannot be maintained on anti-viral therapy due to treatment related toxicity or comorbidities such as renal insufficiency or myelosuppression and at least one of the below:

1. recipients HCT who do not have appropriately restricted donor-derived CMVpp65-CTLs available,
2. solid organ transplant,
3. human immunodeficiency virus,
4. other immunocompromised states,
5. immune competent subjects who require therapy.

Patients will receive adimlecleucel selected from our bank of GMP grade adimlecleucel lines generated from normal HCT donors specifically consented for this purpose. The third party ATA230 to be administered will be selected on the basis of 1) sharing of at least one HLA allele with the patient, and 2) HLA restriction of the CMVpp65CTL by one or more HLA alleles shared by the patient.

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). Adimlecleucel to be infused will be characterized as to immunophenotype, CMVpp65 peptide epitope specificity, HLA restrictions and TCR Vβ usage so as to be able to track disposition, and longevity of these T-cells post infusion.

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 0.5 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 adimlecleucel, patients will 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 adimlecleucel in the blood as measured by quantitation of CTL precursor frequencies, CMVpp65-specific IFNγ+ T-cells (42), or epitope specific-T-cells binding HLA-peptide tetramers and their Vβ usage (43). The third party T-cells will also be monitored by analysis of CMVpp65 IFNγ+ T-cells as to their genetic origin. Patients will also be monitored for any toxicities as described in section 11.0.

4.2 Intervention

The T-cells to be infused will be selected based on criteria mentioned in section 4.0 from our bank of GMP grade adimlecleucel. 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 adimlecleucel /kg/dose/week (with an acceptable range of 0.8 – 1.2×10^6 cells/kg) for 3 weeks or total dosing of 2.4 – 3.6 x 10^6cell/kg per cycle. Patients

will be observed for the following 3 weeks. Additional 3 week courses of adimlecleucel 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 studies 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 HCT. Our studies of the incidence of GvHD in recipients of T-cell depleted matched or 1 allele mismatched HCT 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 (44). 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 (45). Therefore, in the doses of adimlecleucel 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 initial study developed GvHD when treated with transplant donor derived 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 (29). Rooney et al (27), 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. Similarly, in our studies of HCT patients treated with third party virus-specific T-cells for either rituximab refractory EBV lymphoma (N=33) or persistant CMV infection (N=65), a total of 4 patients developed de novo GvHD or exarbation of existing GvHD for 100 days.

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 adimlecleucel 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 from the groups listed below:

1. Normal, HLA typed, CMV-seropositive related and unrelated hematopoietic cell transplant donors who have consented to provide blood or blood white cells to make CMV-specific T-cells for treatment of CMV in the recipient of their transplant and have also specifically consented to the use of their CMV-specific T-cells for another patient, at such time as the transplant recipient is no longer at risk for this complication.

2. Normal, healthy HLA typed, CMV-seropositive volunteer blood donors who consent to provide blood or blood white cells to make CMV-specific T-cells for treatment of CMV infections in transplant recipients whose T cells may add to the HLA diversity of the T cells available in the Adoptive Immune Cell Therapy Bank.

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

Clinical studies for donors are obtained within 1 week of blood donation and include medical history and physical exam, CBC with differential and platelet count. Results of tests must be within a range that would not preclude donating blood or undergoing leukapheresis.

Serologic testing for transmissible diseases is 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: Consenting volunteer blood donors will be requested to provide a leukopheresis.

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

The DCs 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 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 added for 48 hours to induce maturation of dendritic cells. Thereafter, the dendritic cells are 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 Massachusetts) in serum free medium at 37ºC for 3 hours and used for T-cell sensitization as described below.

EBV transformed B cells are generated as described previously (46), 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 (adimlecleucel)

T-cells will be generated as described in published reports and detailed in the appendices (45). 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 HCT 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 adimlecleucel 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 (adimlecleucel)

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 (43, 45).

T-cell cultures providing the required dose of adimlecleucel 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 microbiologically 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 HCT, or marrow or PBSC HCT from seronegative donors, or donors who are either not available or did not consent to provide leukocytes for generation of CMV-specific CTL.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Patients eligible for this trial will be 1) consenting recipients of related or unrelated alloHCT, SOT recipients, or patients with HIV or other immunocompromised states or immune competent subjects who require therapy who 2) 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, and 3 do not have CMV-specific T-cells from the donor of their HCT available.

6.1 Subject Inclusion and Exclusion Criteria

6.1.1 Patient Inclusion Criteria

1. Each patient must satisfy <u>at least one</u> of the following criteria:
   a. The patient has a clinically documented condition associated with CMV (e.g. interstitial pneumonia, hepatitis, retinitis, encephalitis, colitis)
   b. The patient has 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 <u>at least one</u> of the following criteria:
   a. CMV disease is persistent despite ≥ 2 weeks of antiviral therapy or progressing
   c. CMV viremia is persistent or increasing (determined by quantitation of blood CMV DNA) despite ≥ 2 weeks of antiviral therapy
   d. A genetic mutation associated with antiviral drug resistance is present
   b. Unable to continue antiviral drugs due to drug-associated toxicity

3. 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, <u>dysfunctions of these organs will not affect eligibility for this protocol</u> (e.g. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) < 3× the upper limit of normal (ULN) and total bilirubin < 2.5×ULN unless caused by CMV)

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

5. Patient must also satisfy the following criteria:
   a. For subjects who received an alloHCT, the underlying disease for which the alloHCT was performed is in morphologic remission
   b. Availability of appropriate, HLA partially-matched and restricted, adimlecleucel product
   c. Subject or subject's representative is willing and able to provide written informed consent

6.1.3 Patient Exclusion Criteria

1. Patients requiring high doses of glucocorticosteroids (≥ 0.5 mg/kg prednisone or its equivalent)
2. Receiving concomitant investigational therapy (co-enrollment in a non-interventional study or a study for follow-up or sample collection is permitted)
3. Need for high or moderate dose methotrexate or extracorporeal photophoresis for disease control or treatment of active GvHD. Other antimetabolite or T cell directed agents such as azathioprine, tocilizumab or ruxolitinib for control of rejection, autoimmunity or GvHD are allowed but should be discussed with the PI.
4. Antithymocyte globulin or similar anti-T cell antibody therapy ≤ 4 weeks prior to the first dose of adimlecleucel
5. Need for vasopressor or ventilator support
6. Pregnancy
7. Female of childbearing potential or male with a female partner of childbearing potential unwilling to use a highly effective method of contraception
8. Patients with other conditions not related to CMV infection (e.g. uncontrolled bacterial sepsis, other oral infections or invasive fungal infection) which are also life-threatening and which would preclude evaluation of the effects of a T-cell infusion
9. Inability to comply with study procedures
10. Patients who are moribund

7.0 RECRUITMENT PLAN

7.1 Recruitment Procedures

7.1.1 Patient Recruitment

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

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 third-party adimlecleucel 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 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.

8.0 PRETREATMENT EVALUATION

Prior to adimlecleucel 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 28 days of the first infusion. Tests easily repeated (e.g. blood work and chest x-ray) will preferably be performed within 3 days of infusion. Certain tests may be omitted at the discretion of the treating physician:

- Clinical history
- Physical examination 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 DNA in the blood by real-time quantitation PCR
- Chest x-ray (not necessary if CT scan is done)
- GvHD assessment
- Pregnancy test for women of childbearing potential
- Review of concomitant medications:
    - CMV Anti-viral drugs: Foscarnet, Ganciclovir, Cidofovir, Valganciclovir, CytoGam, Brincidofovir, Maribavir, Leflunomide
    - Other CMV treatments: Donor Derived cells
    - Antithymocyte globulin or similar anti-T cell antibody therapy (e.g., OKT3), moderate or high dose methotrexate, or extracorporeal photopheresis.
    - Immunosuppressants: Tacrolimus, Cyclosporine, Sirolimus, MMF, Budesonide, Hydrocortisone, Methylprednisolone/Prednisone (at allowed doses). In addition, azathioprine, ruxolitinib and tocilizumab are allowed if not being used for active GvHD or solid organ rejection and must be discussed with the PI prior to enrollment on study.
- Ophthalmologic exam for CMV retinitis, if clinically indicated
- Colonoscopy for CMV colitis, if clinically indicated
- CT scan, if clinically indicated
- Lymphocyte immunophenotype (BMT short panel) and T cell responses to PHA and CandidaAlbicans, if clinically indicated

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 lymphocyte characterization by flow cytometric phenotyping
- Quantitation of T cells generating IFNγ in response to stimulation with a pool of CMV
- pentadecapeptides
- Quantitation of T cells binding CMV peptide by tetramer analysis in patients expressing HLA alleles for which there are tetramers available
- Analysis of TCR Vβ repertoire

9.0 TREATMENT/INTERVENTION PLAN

This is a single institution phase II trial designed to assess the therapeutic activity of third-party donor-derived CMVpp65 specific T-cells (adimlecleucel) 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 antigen presenting cells, loaded with a pool of overlapping synthetic peptides spanning the sequence of CMV protein pp65.

9.1 Treatment with adimlecleucel

This study will enroll subjects regardless of the underlying susceptibility to CMV, including alloHCT, SOT, human immunodeficiency virus, other immunocompromised states, and immune competent subjects who require therapy. Subjects must have active CMV viremia or disease for ≥ 2 weeks

despite treatment with antiviral therapy or must be intolerant to antiviral therapy due to treatment-related toxicity or comorbidities such as renal insufficiency or myelosuppression.

Subjects will receive adimlecleucel from cell lines generated from normal alloHCT donors or healthy donors, specifically consented for this purpose, based on having a partial match of ≥ 2 HLA alleles shared between the donor and the subject including the HLA restricting allele of the adimlecleucel cell product.

Adimlecleucel will be administered in cycles lasting 5 weeks (35 days). During each cycle, subjects will receive IV adimlecleucel at a dose of 1×10^6 cells/kg (with an acceptable range of 0.8 – 1.2×10^6 cells/kg) for 3 weeks or total dosing of 2.4 – 3.6 x 10^6cell/kg per cycle on days 0, 7, and 14. 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, and then at approximately monthly intervals until 6 months post infusion.

Additional 3 week courses of adimlecleucel may be administered if patients have ongoing disease and/or levels of CMV DNA in blood are still detectable. These secondary courses will usually employ T-cells from the same third party donor. However, adimlecleucel derived from an alternate third party donor's CMV pp65-specific T-cells may be used if no further cells from the same donor are available or for patients with stable or progressive CMV infection refractory to antiviral drugs, since infusion of virus specific T-cells from a secondary donor specific for a different CMV peptide and restricted by the same or an alternate HLA allele shared by the recipient has, in several cases, induced durable regression of disease (23).

Subjects will generally be treated for a maximum of 2 cycles, though subjects achieving PR by the end of Cycle 2 may receive a third cycle. Subjects with PD at the end of Cycle 1 or later, or who experience unacceptable toxicity, will be offered Switch Therapy if appropriate.

SUMMARY OF ACTIONS TO BE TAKEN ON THE DISEASE RESPONSE WITH EACH CYCLE

Response	Action	Notes
Complete Response (CR)	-	-
Partial Response (PR) / Stable Disease (SD)	If available, administer another cycle of adimlecleucel from the same cell line and reassess	Maximal response is a PR after 3 consecutive PR responses; after maxima l response (3 cycles resulting in PRs), then observe
Stable Disease (SD)	If SD is the first Cycle response, administer the same adimlecleucel cell line for the next cycle and reassess; if SD is the second Cycle response, administer a different adimlecleucel cell line from a different donor (Switch Therapy) for the next cycle and reassess	If the subject has SD after 2 cycles of the same adimlecleucel cell line, a different adimlecleucel cell line may be administered (Switch Therapy)

Progressive Disease (PD)	Subsequent Cycles of adimlecleucel with different restricting HLA alleles from a different donor (Switch Therapy)	If Switch Therapy results in CR/PR/ SD continue with subsequent cycle of adimlecleucel from the same donor, then observe. If Switch Therapy results in PD, discontinue therapy.

All subjects receiving antiviral drugs can remain at their current dose during the adimlecleucel treatment period. Subjects may also continue treatment with rapamycin and calcineurin inhibitors. Corticosteroid doses should be reduced to the lowest acceptable dose; adimlecleucel has not been administered with prednisone-equivalent daily doses > 0.5 mg/kg.

9.2 Subjects

Subjects will include patients treated with adimlecleucel derived from third party donors. This will include recipients of allogeneic hematopoietic cell transplant recipients, solid organ transplant (SOT) recipients, human immunodeficiency virus, other immunocompromised states, and immune competent subjects with persistent CMV infection or viremia.

9.3 Selection of Adimlecleucel

Patients will receive adimlecleucel selected from our bank of GMP grade adimlecleucel lines generated from normal HCT donors specifically consented for this purpose. The third party adimlecleucel to be administered will be evaluated on the basis of 2 criteria: 1) they share at least one HLA allele with the patient, and 2) they are restricted by at least one HLA allele shared by the patient.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

The schedule of tests described should be adhered to whenever possible. However, in c ertain 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:

10.1 Treatment Cycle

10.1.1 Treatment days

Patients will have vital signs assessed prior to each infusion, immediately after the infusion, and at hours 1, 2 and 4 after each infusion (+/-) 10 minutes.

On Days 0, 7 [± 2 days], and 14 [± 2 days] of each cycle, perform the following assessments: prior to the infusion.

- History, Review of Systems and Physical Exam (including GvHD assessment for cycle 1 only. For patients receiving more than 2 cycles, GvHD assessment will occur at the start of each cycle only.).

- Assessment of SAEs and anti-viral and immune suppressive concomitant medications
- Assessment of CRS and ICANs per institutional standards
- CMV DNA quantitation by PCR

10.2 Follow-up

10.2.1 Monitoring during the first 8 weeks

Patient will be monitored for eight weeks after administration of the first dose of adimlecleucel. During this period, perform the following:

- Assessment of SAEs and concomitant medications
- Assessment of CRS and ICANs per institutional standards
- CMV DNA quantitation by PCR to determine if an additional cycle is needed
- Assessment/Phone assessment for symptoms of GvHD weekly at baseline and prior to each infusion, week 5 (Day 28 – 32) if patient not receiving subsequent cycles of CMV-CTLs
- The timing of Consensus GvHD assessments is dependent on the number of infusions patients receive and will be performed as per institutional standards.

For patients who receive subsequent cycles of cells, the monitoring calendar will re-start with the first dose of the subsequent cycle, with the exception of GvHD assessments, which will occur as above.

10.2.2 Safety follow-up 30-35 days after the last dose

The safety follow-up visit should occur at the center 30-35 days after the last dose of adimlecleucel. At this visit, the following is required:

- Assessment of SAEs and concomitant medications
- Assessment of CRS and ICANs per institutional standards
- CMV DNA quantitation by PCR to determine if an additional cycle is needed

10.2.3 Follow-up at 90 and 180 days after the last dose

Follow-up visits will be performed at 90 (± 14) days and 180 (± 30) days after administration of the last dose of adimlecleucel. At these visits, perform the following:

- CMV DNA quantitation by PCR
- Survival status
- Consensus GvHD assessments as per institutional standards.

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

Evaluation	Prior to CMV CTLs	Infusion 1	Infusion 2	Infusion 3	Wk 3	Wk 4	Wk 5	Week 6-7 [ab] / Safety Follow-Up 30-35 days after last dose	Wk 8	Mo 3 Follow-up 90 ± 14 days after last dose	Mo 6 Follow-up 180 ± 30 days after last dose
		Day 0	Day 7 (± 2 days)	Day 14 (± 2 days)	Day 21	Day 28	Day 35	Day 44-49	Day 56		
ICF signed	X										
Inclusion/exclusion criteria	X										
Medical history	X										
Weight	X										
Hematology and serum chemistry[c]	X										
Pregnancy test [d]	X										
High resolution HLA typing for subject [e]	X										
Chest X-Ray [f]	X										

Vital signs	X	X[g]	X[g]	X[g]							
ATA230 Treatment [h]		X	X	X							
Physical Exam	X	X	X	X				X		X	X
Concomitant medications	X	X	X	X	X[b]	X[b]	X[b]	X	X[b]	X[b]	
CMV DNA by PCR [i]	X	X	X	X	X	X	X	X	X	X	X
SAEs [j]	X	X	X	X	X	X	X	X	X		
GvHD [k]	X	X	X	X			X[b]				
Consensus GvHD CRS and ICANS [l]							X		AS PER INSTITUTIONAL STANDARDS		
Survival status										X	X
Evaluations below are to be performed as clinically indicated:											
Physical Exam					X	X	X		X		
Hematology and serum chemistry[c]	X	X	X	X	X	X	X	X	X	X	X
CT Scan	X										
Ophthalmology scan, Colonoscopy, Cultures of affected body fluids or tissues	X	X	X	X	X	X	X	X	X	X	X
ECHO/EKG	X										
T cell immunophenotype short panel	X			X				X		X	X
Mitogens (PHA)				X				X		X	X
Antigen Specific Proliferation	X			X				X		X	X

[a] The Safety in-person follow-up visit will overlap with the weekly follow-up visit during week 6 or 7. Evaluat e CMV DNA weekly during both weeks. Perform assessment of CRS and ICANs per institutional standards to align with FACT requirements

[b] Evaluation may be completed by phone

[c] CBC & CMP

[d] For women of childbearing potential

[e] In addition, as available through medical records, high resolution HLA typing of the allograft donor for alloHCT recipients or low resolution HLA typing of the organ donor for SOT recipients

[f] Per section 8, not necessary if CT scan is done

[g] On each dosing day, monitor vital signs prior to the infusion, immediately after the infusion, and at 1, 2 and 4 hours (± 10 minutes at each time point) following initiation of the infusion

[h] If patient is given additional cycles of ATA230, 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

[i] 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, and then at approximately monthly intervals until 6 months post infusion

[j] Any adverse events observed at screening, should be included as medical history

[k] GvHD evaluations to be performed at pretreatment, baseline (D1) and weekly (D7 and D14) for the first 3 infusions. Then week 5 (Day 28 – 32). For patients receiving more than 2 cycles: baseline at the start of each cycle.

[l] GvHD consensus to be performed on Day 30 from the first infusion and at 100 (± 10) days post first CMV CTL infusion of last cycle

11.0 TOXICITIES/SIDE EFFECTS

One of the primary objectives is to continue to identify the risk of development or flare of pre-existing graft versus host disease.

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 allogeneic 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). In patients treated with third party-derived virus-specific T-cells, this risk could be increased. 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. Patients who might subsequently receive donor lymphocytes, boosts, or a secondary transplant will be evaluable up until that time point.

In Protocols 95-024, 05-065, 11-130, and 12-086, and 14-070 of subjects are evaluated for the occurrence of GvHD for 100 days. A total of 4 patients have developed de novo GvHD one after EBV-CTLs and 3 after CMV-CTLs. GvHD in these patients was as skin GvHD in 3 patients and gastrointestinal GvHD in 1 patient. Two cases were considered unlikely related to third party viral specific CTLs and two possibly related. An additional 11 subjects, with a history of GvHD, had GvHD after administration CTL administration; these cases were considered unrelated or unlikely related to CMV-CTLs.

In this study, we will be capturing and tracking Grade 3-5 toxicities which occur within 30 days following an infusion of adimlecleucel and are potentially attributable to treatment on study. Toxicities which are attributable to underlying malignancy and/or were present prior to initiation of therapy will not be tracked. We will perform assessment of CRS and ICANs per institutional standards to align with FACT requirements. Please see section 17.2 for Serious Adverse Event reporting.

11.1 Identified risks

1. **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.
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 may be at increased risk for development of pulmonary toxicity including diffuse alveolar hemorrhage.

3. **Graft versus Host Disease.** See section 11.0
4. **Hypoxia.** Hypoxia was reported as an SAE in 15 of 80 subjects (18.8%) treated with CMV-CTLs in Protocols 05-065, 12-086, and 14-070 (through the safety data-cut date of 29 April 2016). None of the SAEs were considered related or likely related to study product; 4 were considered possibly related and 11 were considered unlikely or unrelated. In general, CMV pneumonia was the most common underlying cause of hypoxia, though in many cases, clear alternate etiologies were present, including *Pneumocystis jirovecii* pneumonia, parainfluenza, pulmonary embolus, botulinum infection, pulmonary hemorrhage, sepsis, adenovirus pneumonia and fluid overload. Two cases were reported as possible local inflammatory response related to CMV-CTLs (at anatomic site of CMV antigen expression) as playing a role in hypoxia. The possibility that a cell-mediated response could make CMV pneumonia worse is considered a risk of therapy. CMV pneumonia appeared to be a final common pathway for the resistant CMV infection in a number of subjects for whom multiple antiviral therapies had failed

   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.

5. **Allograft Rejection. Similar to the concern for GvHD** 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 allogeneic 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 Allograft rejection. Allograft rejection was reported in one subject treated on a Single Patient Use IND. The subject experienced renal allograft cellular rejection which was determined to be possible related to adimlecleucel.

11.2 Theoretical risks

1. **Acute Toxicities. Allergic Transfusion Reactions, and Cytokine Release Syndrome.** 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. Similarly our prior experience and that of other centers predict that the risk of cytokine release syndrome which is characterized by fever or an allergic reaction to the cell infusion manifested by is low. Patients developing fever after infusion of ATA230 will be assessed for other evidence of cytokine release syndrome and treated for it as per institutional guidelines.
2. **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.
3. **Marrow Allograft Rejection.** Patients receiving third party donor-derived CMV specific T-cell infusions for treatment of CMV infection may be at risk of an acute rejection episode following infusion of the T-cells. This risk is expected to be very low, since these T-cells only transiently engraft. Indeed, rejections have not been observed in either marrow or organ allograft recipients

treated with third party EBV-specific T-cells (31, 41). Such episodes could be precipitated if significant numbers of alloreactive T-cells are present in the T-cell inoculum. In allogeneic hematopoietic cell transplant recipients, this could lead to loss of the hematopoietic cell transplant, resulting in marrow aplasia, a potentially lethal complication. Therefore, if severe leukopenia or thrombocytopenia is observed that is potentially attributable to the infused T-cells, the patient will be treated with prednisone and/ or anti-thymocyte globulin to eliminate the infused third party T-cells.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary endpoint of this study is to assess whether the infusion of adimlecleucel has therapeutic activity in curing CMV infections in recipients of HCT, SOT recipients, HIV, other immunocompromised states or immune competent subjects who require therapy. This study specifically will evaluate the therapeutic efficacy and persistence after infusion of adimlecleucel derived from third party donors for the treatment of CMV infections. The second clinical end point of this study is safety, especially the evaluation of GvHD induction after infusion of the third party adimlecleucel.

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. Final responses will be captured in CRDB and scored between days 28-42 post the final infusion of each cycle. Such window of evaluation ends the first day of the following cycle if patient switches cell lines and overlaps days 28-42. 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.

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. For patients with a baseline CMV PCR < 13,800 copies/mL a partial response will be defined as having a CMV PCR of <137 copies/mL on two occasions during the assessment period.

Stable disease will be defined as no change in the clinic severity of disease in any organ and CMV DNA is decreasing by the time of the evaluation time point but may be higher than the baseline CMV PCR (reflecting an increase that can occur during in vivo expansion of CMV-CTLs) and has not decreased by $2xLog_{10}$.

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

Patients lost to followup either during therapy or followup or patients with changes in antiviral therapy during treatment with adimlecleucel such that any changes in the extent of CMV viremia or disease cannot be attributed to cellular therapy will be defined as not evaluable for outcome assessment. Patients evaluable for some but not all cycles of cellular therapy will be evaluated as such.

Graft versus Host Disease initially developing 10 to 100 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 to 100 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:

- Withdrawal of consent by subject
- Occurrence of an AE that precludes further participation
- Subject chooses to take a prohibited treatment, such as use of a different investigational medication or device
- Refusal of the patient ot continue treatment observations
- Significant noncompliance on the part of the patient
- Development of another disease (e.g. Adenovirus infection) that 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
- Lost to follow-up

Patients who received a concomitant antiviral therapy due to toxicity of the current therapy or occurrence of another viral infection will be replaced. Data regarding safety of the CTLs will still be collected for those patients.

14.0 BIOSTATISTICS

A phase 2 trial design will be applied to determine the efficacy and safety of adimlecleucelfor the treatment of marrow transplant patients with CMV. The endpoint of this study is complete response or partial response, as defined in Section 12. It is anticipated that accrual will be completed within 3 years.

The study design for HCT recipients aims to detect between response rates of 0.40 and 0.56 in the population. This two-stage design has a target sample size of 69 evaluable patients. If 15 or fewer patients respond in the first 36 patients that are evaluable for outcome assessment, then the trial will be stopped. If at least 16 patients respond, then 33 additional patients will be accrued for therapeutic response assessment. At the conclusion of the trial, if 32 or fewer patient responses are observed, we will conclude that the treatment is not sufficiently active. This design has a power of at least 0.90 for a population CR/PR rate equal to 0.56 using a one-sided test with type 1 error rate of 0.10. To reach 69 evaluable patients, it is anticipated that 81 patients will be recruited.

Based on the limited data in the setting of SOT recipients as well as immune deficient and immune competent individuals, results for these patients will be analyzed separately and will be descriptive.

In order to reduce patient risk, the study design includes early termination in the event of excessive graft versus host disease or solid organ rejection, requiring addition of immunosuppressant therapy to doses above those at the time of enrollment on trial, during the accrual period. The calculation is based on a group sequential design in a single-arm trial with a binary outcome.

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
Or solid organ transplant rejection	4 within the first 24 patients 5 within the first 35 patients 6 within 51 patients	0.20	0.90

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 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 that the participant is eligible to enroll in the study. Study staff is 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).

15.2 Randomization

There is no randomization in this study.

16.0 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 throughout 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).

16.1 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.

16.2 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.

17.0 PROTECTION OF HUMAN SUBJECTS

17.1 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.

17.2 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 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

18.0 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.

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