Administration of Virus-Specific Cytotoxic T-Lymphocytes for the Prophylaxis and Therapy of Adenovirus Infection post Allogeneic Stem Cell Transplant

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CHECKLIST FOR PATIENT ELIGIBILITY AND NECESSARY INFORMATION

Patient ID ____________________________________________________

Patient Name__________________________________________________

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
<th>VALUE/DATE</th>
</tr>
</thead>
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Any “NO” answers will make a patient ineligible for study participation

____  ____  ______  Recipients of allogeneic donor stem cell transplants.

____  ____  ______  Minimum 50% donor chimerism in either peripheral blood or bone marrow or relapse of their original disease.

____  ____  ______  No evidence of GVHD > Grade II at time of enrollment

____  ____  ______  Life expectancy > 30 days

____  ____  ______  Lansky/Karnofsky scores > 60

____  ____  ______  No severe intercurrent infection

____  ____  ______  Not receiving Cidofovir or other antiviral therapy for Adenovirus infection.

--------  ------  ---------  Patient has not received other viral specific CTL prophylactically within 4 weeks of receiving Adv-CTL.

____  ____  ______  Absence of severe renal disease (Creatinine > 3X normal for age)

____  ____  ______  Absence of severe hepatic disease (direct bilirubin > 3mg/dl, or SGOT > 500)

____  ____  ______  No evidence of Adenoviral disease prior to day +30.

____  ____  ______  Patient/Guardian able to give informed consent.

Signature of MD ____________________________________________________

Date______________________________________________________________

To check eligibility of a patient, call Dr. Bollard on 832-824-4781.
1.0 OBJECTIVES

1.1 To determine the safety, toxicity and maximum tolerated dose (MTD) of one intravenous injection of donor-derived adenovirus-specific cytotoxic T lymphocytes (CTLs) given as adenovirus prophylaxis to patients at risk of developing adenovirus infection after allogeneic stem cell transplant.

1.2 To evaluate the recovery of virus-specific immunity after CTL infusion and assess its correlation with protection from viral load and disease.

1.3 To obtain preliminary information regarding whether the presence of antigen is required for Ad-specific CTL persistence in vivo.

2.0 BACKGROUND AND RATIONALE

2.1 ADENOVIRUS

Adenoviruses (Ad) are non-enveloped, lytic, DNA viruses capable of infecting most animal species. Pathogenicity varies according to group and type, and although acute infection is sometimes severe, it is rarely fatal in otherwise healthy adults. Initial infection ultimately results in cell destruction. However, Ads do persist and can be detected months after primary exposure. Humans are susceptible to infection with 51 serotypes of Ad, forming six distinct groups (A to F); Ads generally infect mucosal epithelium, but the serotypes differ in their tissue-specificity and virulence. Following viral infection, transcription is temporally regulated, beginning with immediate early genes encoded in the E1A region, followed by the delayed early units, E1B, E2, E3, E4 and L1. About a third of the viral genome is devoted to counteracting the antiviral immune response. These viral immune evasion strategies ensure prolonged survival of infected cells and facilitate virus transmission. The early expression of immune evasion genes is likely to inhibit immunity to the majority of the early and late antigens. For this reason our strategy is to target immunodominant virion proteins that access the endosomal/lysosomal pathway and cytosol by receptor-mediated endocytosis and endosomolysis and are then presented to the immune response before immune evasion functions are expressed.
2.2 Adenovirus infection in stem cell transplant recipients

The pathogenicity of Adenovirus varies according to group and type and, although acute infection is sometimes severe, it is rarely fatal in otherwise healthy adults. Thus, in normal individuals, the antiviral immune response of the host controls the extent of virus replication and spread. However, Ads are one of a group of opportunistic pathogens of immunosuppressed patients, where severe, prolonged and even fatal infections are common. The frequency of severe Ad disease is increasing in association with growing numbers of immunocompromised individuals, and fatality rates as high as 50% to 80% have been reported. While there are anecdotal reports of adenoviral disease responses to Cidofovir, there are no approved antiviral agents with proven efficacy, nor are there currently any prospective randomized controlled trials of potentially useful anti-Ad therapies.

Up to 27% of allogeneic stem cell recipients develop detectable Ad in one or multiple sites. This incidence increases in high-risk recipients who receive T cell-depleted stem cells from unrelated or HLA-mismatched related donors. About 70% of those with detectable Ad in serum develop fatal Ad-associated disease associated with hemorrhagic cystitis, pneumonia, nephritis, hepatitis, colitis, and pancreatitis. Treatment options for adenoviral disease are limited, with anecdotal reports of success using ribovirin, ganciclovir and cidofovir and, intriguingly, donor leukocyte infusions. SCT recipients succumb to a range of Ad serotypes, most commonly 7, 11, 34, and 35 from group B and types 2 and 5 from group C have been detected.

2.3 Adoptive Immunotherapy for viral infections post transplant

Virus-specific CTL have proven successful in the prevention and treatment of EBV and CMV-associated diseases in hematopoietic stem cell transplant (SCT) recipients. Our group has considerable experience with the adoptive transfer of donor-derived EBV-specific CTLs to prevent and treat EBV-associated lymphoproliferation (EBV-LPD) post transplant. EBV specific CTL were generated from the stem cell donor using autologous EBV transformed lymphoblastoid cell lines (LCL) as antigen presenting cells. The CTL were transferred to recipients at high risk of developing EBV-LPD. Over 60 patients received CTLs prophylactically. Nine of these patients had evidence of incipient EBV lymphoma prior to CTL infusion. 3 patients developed local inflammation during a therapeutic response with one developing adenoid enlargement with necrosis, one developing fever and a LLL pulmonary
infiltrate and the third a transient elevation in transaminases. None of the 60 patients in the prophylaxis study developed EBV lymphoma compared to an incidence of 11.5% in patients not receiving prophylaxis.16

CMV prophylaxis with adoptively transferred, donor-derived CMV-specific CTL was first explored by Walter et al. When CD8+ T cell clones were transferred to recipients of matched sibling grafts, there were no adverse events, CMV specific immune responses were reconstituted and the patients were protected from developing CMV disease17. A clinically more feasible approach was taken by MacKinnon et al. who generated CMV-specific CD4 and CD8+ T cells using dendritic cells pulsed with CMV Ag derived from a CMV-infected human lung fibroblast cell line as the antigen presenting cell.18,19 Patients were monitored for CMV reactivation and T cell lines were infused at the time of first PCR reactivity. There were no infusion related toxicities and none of the 13 patients treated with a single dose of 10^5/kg CMV CTL, developed CMV disease.

2.4 Potential of Adoptive Immunotherapy for Adenovirus

Since it is possible to transfer cell-mediated responses to EBV and CMV, we wish to evaluate this approach in patients with Ad infection by infusing donor-derived, Ad-specific CTL. Immune intervention for virus-associated diseases initially requires an understanding of the immune control of the virus in a healthy individual. In order to develop an effective and protective therapy, it is important to (i) determine if the T cell response is polarized against certain adenoviral antigens, and (ii) if there is a hierarchy of immunodominance among these proteins. Ad has developed mechanisms to evade the cellular immune response; the virus encodes three gene products that facilitate persistence by antagonizing the antiviral responses of the host; E1A proteins and VA RNAs, which inhibit the cellular response to interferon-α and −β, and E3-coded products that protect infected cells from killing by CTLs and tumor necrosis factor (TNF). Since these proteins are expressed early during transcription it is likely that the other early and all late proteins are subsequently protected from immune surveillance. This suggests that the Ad-specific T cell response may be dominated by specificity against epitopes in the input viral capsid, a protein shell, composed predominantly of hexon, penton and fiber, which surround the DNA-containing core. In pre-clinical studies we have characterized the phenotype, function and antigen-specificity of Ad-specific CTLs generated using Ad-transduced PBMCs as the first and second stimuli, and Ad-transduced LCLs for subsequent restimulation and expansion. We have demonstrated that
monocytes in the PBMC mix are capable of being infected by the Ad5f35 vector and consequently of reactivating Ad-specific memory T cells while autologous Ad-transduced LCLs are used to expand the reactivated T cell population. We can generate CTLs containing a CD4 and CD8-specific component using this method and have been successful in identifying the hexon protein as a dominant T cell target antigen.\textsuperscript{20}

To facilitate immunological monitoring we have identified two novel hexon-specific CD8+ T cell epitopes restricted through HLA A1 and A24 alleles, and have made the relevant tetramers.\textsuperscript{21} In addition an immunodominant CD4 peptide that is both cross-reactive and promiscuous, has already been identified in the hexon protein by Olive and colleagues.\textsuperscript{22-23} We are currently in the process of identifying further CD4 and CD8 epitope peptides.

The success of this treatment rests on the hypothesis that the CTL raised will provide cross-reactive protection against different Ad serotypes and lack of CTL cross-reactivity between Ad serotypes would be a potential problem. However, our pre-clinical studies show that CTL generated using this method readily recognize representative adenovirus serotypes from the five different subgroups, suggesting that a CTL line generated in vitro using an Ad vector from a sub-group C virus will be capable of expanding T cells that can recognize many if not all of the Ads that could be encountered in a clinical setting.

2.5 Rationale for Current Protocol

The goal of our study is to generate Ad-specific cytotoxic T-cells and adoptively transfer them as Adenoviral prophylaxis to patients at risk of adenoviral infection after allogeneic stem cell transplant. These are patients of any age who receive either a T cell depleted transplanted from a matched/mismatched related donor or a matched or mismatched unrelated donor (MUD) or if patients have received an anti T cell antibody such as Campath or ATG in conditioning. The indications for transplant will include pre-malignant and malignant diseases, aplastic anemia, and immunodeficiency syndromes. One dose of Ad-specific CTL will initially be given from 30 days after transplant and toxicity will be assessed by standard NIH criteria. Antiviral prophylaxis for adenovirus will not be used, but patients will be monitored for Ad reactivation by screening blood, stool, and urine by adenoviral PCR assay.

Secondary endpoints are:
- to estimate the effective cell dose of Ad-specific CTLs that prevents Ad reactivation in this patient population.
- to evaluate reconstitution of virus-specific immunity in patients after CTL infusion and its correlation with protection from viral reactivation/disease.
- to evaluate the efficacy of Ad-specific CTL in reducing the presence of adenoviral positivity in stool, blood, or urine as detected by PCR or culture.
- to evaluate whether persistence of CTL is dependant on the presence of adenoviral infection

2.6 Risks of administering adenovirus-specific CTL

Over 60 patients have been treated on our previous adoptive immunotherapy protocol in which allogeneic EBV-specific CTL were administered after bone marrow transplantation. We have not observed any immediate complications that could be attributed to CTL infusions. The main complication associated with infusion of EBV CTL was an inflammatory response in a patient with bulky EBV lymphoma. This would also be a potential risk in patients with adenoviral pneumonia or hepatitis. However both these disease states have a high mortality with no other available treatment.

As the CTL are donor derived, graft-versus-host disease (GVHD) is a potential side effect. Although we will screen CTL lines for reactivity against other host tissues such as lymphoblasts there is no completely reliable in vitro assay for excluding this possibility. We will take additional precautions to minimize this risk. First, any patient with preexisting GVHD of > Grade 2 will be excluded from the study. Second, we will administer CTLs at a maximum dose of $1.35 \times 10^8$ cells/m$^2$. This is a smaller number of T cells than administered at the time of an unmanipulated marrow infusion.

None of the patients treated with CMV-specific CTL by Walter et al developed GVHD. In the cohort of patients treated by MacKinnon et al., 3/13 patients developed mild (Grade I) GVHD. Since immunosuppression had been withdrawn early in this study, it is unclear if this side effect was due to CTL infusion. In our studies with EBV specific CTLs no patients developed de novo GVHD and only 2 of 60 had recurrence of previously documented GVHD. Should any patient develop GVHD, the site will be biopsied and examined histologically to confirm the clinical diagnosis.

Another potential hazard is the infusion of EBV transformed B cells which have been cocultured with the CTL during generation of the T cell lines. This is unlikely to constitute an
additional risk to the recipient for several reasons. First, the lymphoblastoid cells (LCL) are not viable because they have been irradiated with 4000cGy and co-cultured with known effectors. In addition, we will add acyclovir to the LCL cultures so that no productive virus will be present in these cultures. Finally we will monitor levels of EBV DNA in peripheral blood by PCR pre- and post CTL infusions. Transfer of EBV or infusion of EBV-transformed B cell lines has not been a problem in over 60 patients who have received T cells that have been co-cultured with irradiated LCLs in our studies. Nor have we detected the B95-8 strain of EBV in any of our CTL recipients with measurable peripheral blood virus load.

Another potential hazard is the infusion of cells infected with the adenoviral vector (Ad5f35), which could lead to an inflammatory reaction. The infusion of adenoviral infected cells is however unlikely to constitute an additional risk for several reasons. First, the Adenoviral vector will be RCA negative. Secondly, in this study we are initially using the adenoviral vector to infect peripheral blood mononuclear cells to stimulate the T cells. We have shown that only CD14+ve cells (monocytes) and not T cells or B cells become infected with the adenoviral vector at the MOI we use. Subsequent stimulations of the T cells will be with lethally irradiated monocytes and then with EBV-lymphoblastoid (B cell) lines transduced with the Ad5f35 vector. Prior to stimulation, the irradiated LCL and monocytes will be washed 3 times which we have shown eliminates 4 logs of free adenovirus from the cultures. Thirdly, Ad-specific CTL will be cultured for at least 7 days after the last stimulation with LCL infected with the Ad5f35 vector since pre-clinical studies have shown that this ensures no Ad-infected LCL remain alive to be administered to the patients. Finally, we will not infuse any T cells which contain >2% CD19 positive cells (B cells) or >2% CD14 positive cells (monocytes).

Unlike CMV and EBV, which are persistent viral infections, adenoviruses produce only transient infection. This may have a substantial impact on the survival and expansion of the infused cells. Unlike CTL specific for EBV and CMV, these adenovirus specific CTL may not survive in vivo since antigenic exposure may be absent in uninfected patients. Hence one of the aims of this study is to assess the effects of adenovirus infection on CTL survival and expansion in vivo. While patients will receive a single dose of adenovirus specific CTL regardless of their adenoviral status, a second dose of CTL will be administered to patients who become adenovirus infected 4 weeks or more after the initial dose of CTL. Adenovirus infection will be defined as the presence of adenoviral positivity as detected by PCR or...
culture from ONE site such as stool or blood or urine or nasopharynx. Adenovirus disease will be defined as the presence of adenoviral positivity as detected by culture from more than two sites such as stool or blood or urine or nasopharynx. The functionality of this second dose compared to the behavior of the first will provide important information about the correct timing of AdCTL administration after stem cell transplant. Since no effective alternate treatment for adenoviral infection exists (in contrast to CMV) this protocol should not compromise patient safety.

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

- Recipients of allogeneic (i.e. HLA matched or mismatched related or unrelated) donor stem cell transplants at risk for Adenoviral disease.
- No evidence of GVHD > Grade II at time of enrollment.
- Life expectancy > 30 days
- No severe intercurrent infections
- Lansky/Karnofsky scores >60
- Absence of severe renal disease (Creatinine > x 3 normal for age)
- Absence of severe hepatic disease (direct bilirubin > 3 mg/dl or SGOT > 500)
- Patient must be at least 30 days post transplant to be eligible to receive CTL.
- Not receiving Cidofovir
- Patient has not received other viral specific CTL prophylactically within 4 weeks of receiving Adv-CTL.
- Patient/guardian able to give informed consent

3.2 Exclusion Criteria

- Patients with GVHD Grades III-IV
- Patients with hepatic or renal disease as specific above
- Patient has received other viral specific CTL (e.g. EBV-specific CTL or CMV-specific CTL) within 4 weeks of receiving Adv-CTL.
• Patients with Adenoviral disease prior to day +30 post transplant. Adenoviral diseases defined as the presence of more than two sites positive for adenovirus by culture
• Patients with less than 50% donor chimerism in either peripheral blood or bone marrow or patients with relapse of original disease

3.3 Inclusion Criteria for DONORS:
• Donors for allogeneic (i.e. HLA matched or mismatched related or unrelated) stem cell transplants who have fulfilled eligibility for and consented to stem cell donation as per the stem cell transplant program’s standard operating procedures (SOPs F03.04.2 Donor selection, F03.01.2 Donor evaluation and F03.05.2 Donor Deferral).
• Patient/guardian able to give informed consent

4.0 STUDY DESIGN
This protocol will be discussed with eligible patients and, when appropriate, their guardians, and informed consent for participation of the study will be obtained. Enrollment of eligible patient/donor pairs will commence 4-5 weeks prior to stem cell transplant as it will take up to 6 weeks to prepare EBV-LCLs and Ad CTLs. The National Marrow Donor Program (NMDP) will have a NMDP IRB protocol and consent form that will be used when obtaining blood for cell line preparation from an unrelated donor.

Only patients who receive their care in the integrated Cell and Gene Therapy Transplant program at Texas Children’s Hospital and Methodist Hospital are eligible for this study.

All cell culture and gene transfer manipulations will be carried out in the Center for Cell and Gene Therapy GMP facility using current standard operating procedures (SOPs). After quality assurance testing is complete a certificate of analysis will be issued.

4.1 Transduction of PBMCs with an Ad5f35 Vector
50-100 ml of peripheral blood will be collected from the stem cell donor (subjects must be at least 12 kg or 26 pounds). PBMCs will be transduced with the recombinant Ad5/35 at 200 vp/cell.
4.2 **EBV Transformed Lymphoblastoid Cell Line Preparation**

5-20 ml of the peripheral blood collected from the donor will be used for the establishment of an EBV transformed lymphoblastoid cell line (EBV-LCL) by infection with virus produced from the B95-8 master cell line according to our SOP. The EBV-LCL will take approximately 4-6 weeks to generate.

4.3 **Transduction of LCL with Ad5/35**

The adenoviral vector will be used to infect LCL ex vivo to stimulate Ad-specific CTLs which are the product that will be administered to the patient. These LCL will be transduced with the recombinant Ad5/35 at 500 vp/cell 1 day prior to each CTL stimulation, and used to expand Ad-specific CTL after two PBMC stimulations. The recombinant adenoviral vector (Ad5/35) is an E1, E3 deleted Ad5 vector in which the fiber protein from Ad serotype 35 has been substituted. Importantly, the Ad5f35 vector, which we will use, does not contain any transgene.

4.4 **Cytotoxic T-Cell Line Preparation**

Ad-specific CTL are stimulated by direct PBMC infection on day 0, restimulation with Ad-infected, irradiated PBMC on day 8 to 10, then weekly restimulation with Ad-infected LCL. IL-2 will be added twice weekly from day 23 until sufficient CTL have been expanded for the patient dose and safety testing. The frequency of antigen-specific CTL will be determined using tetramer reagents, if available. The functional specificity of the CTL will be tested in patient (allogeneic) LCLs infected with Ad5/35 as stimulators in an ELISPOT assay.

After establishment, the CTL lines will be checked for identity, phenotype and microbiological culture and cryopreserved prior to administration according to SOP. Release criteria for administering the CTL to patients include viability >70%, negative culture for bacteria and fungi for at least 7 days, endotoxin testing < 5EU/ml, negative result for Mycoplasma, <10% killing of autologous lymphoblasts at a 20:1 ratio, <2% CD19 positive B cells <2% CD14 positive monocytes and HLA identity.

4.5 **Generation of recipient lymphoblasts**
10-40mL of blood from the recipient will be obtained prior to transplant for the generation of lymphoblasts, which will be used as targets against which to test the cytotoxicity specificity of the CTL lines. CTL lines should not kill recipient lymphoblasts. PBMC will be stimulated with an agonistic antibody to CD3 (OKT3) at 100 ng per mL, then expanded with IL-2 for two weeks or until sufficient cells have been obtained to freeze at least three vials at 5 x 10^6 cells per vial.

4.6 Administration and Monitoring

4.6.1 Patients will be evaluated in the clinic. Patients will be monitored for clinical toxicity by the NCI Common Toxicity Criteria Scale (Version 2.0 located at <http://ctep.cancer.gov>). Patients will be evaluated for development of GVHD during routine follow-up evaluations in the clinic.

4.6.2 Virus load will be measured both by culture and in serum by conventional PCR assay and later on by quantitative real time PCR when this assay has completed validation studies in our laboratory. Any positive blood samples will be screened for the presence of the Ad5f35 vector. We have extensive experience with virus load monitoring in immunosuppressed patients before and after CTL infusion. Virus identification will be performed by the clinical laboratories at Texas Children’s Hospital. If there is evidence of an infection with RCA from the Ad5f35 vector then the trial will be stopped and FDA notified.

4.6.3 Immunological monitoring. To measure the effect of CTL infusion on immune responses to Ad the frequency of T cells secreting Th1 (γ-IFN) will be compared before and after T cell infusion after pulsing PBMC with Ad vectors. Additional tests may be performed as additional tetramers are developed.

5.0 TREATMENT PLAN

5.1 Cell Administration
Cell administration will be as follows:
Adenovirus specific T cells will be thawed and given by intravenous injection. This is a Phase I dose escalation study of one infusion of Adenovirus-specific CTL given to patients at risk for Adenoviral disease after matched or mismatched unrelated or
matched or mismatched related donor stem cell transplant. Four dose levels will be explored. The lowest level will be 1x dose of $5 \times 10^6$cells/m$^2$ and the highest will be 1x dose of $1.35 \times 10^8$/m$^2$. Cohorts of size 2 will be used at each dose level (depending on toxicity) using the modified continual reassessment method (CRM) described below. If there are no toxicities and immunological efficacy is not seen at any dose, then the doses will be further escalated after additional local and federal approval. If the patient develops adenoviral infection as defined in section 2.6, they will be eligible to receive one additional injection of CTLs at the original dose.

### 5.1.1 Dose Levels and Dosing Schedule

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>CTL Dose Given from Day +30 post SCT</th>
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<tbody>
<tr>
<td>1</td>
<td>$5 \times 10^6$/m$^2$</td>
</tr>
<tr>
<td>2</td>
<td>$1.5 \times 10^7$/m$^2$</td>
</tr>
<tr>
<td>3</td>
<td>$4.5 \times 10^7$/m$^2$</td>
</tr>
<tr>
<td>4</td>
<td>$1.35 \times 10^8$/m$^2$</td>
</tr>
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</table>

### 5.2 Escalation of Adenovirus-specific CTL infusions

Initially, Adenovirus-specific CTL will be given on or after day 30 post transplant. If patients have Adenoviral infection (as defined as culture/PCR positive from one site) at their 30 day or subsequent evaluations post CTL, they are eligible to receive one additional dose of CTLs (given no less than 2 weeks apart) at the same dose of the first CTL providing the patients meet eligibility criteria described in section 3.0.

A modified CRM design will be employed as described in section 8.0 below. In general, cohorts of size two will be used starting at the lowest dose level. The logistic function is employed to model the dose-toxicity curve. The CRM repeatedly updates the dose-toxicity curve and subsequent patients are accrued at the dose level with an associated
probability closest to the target acceptable toxicity rate equal to 20%. MTD is defined as the dose which causes DLT in at most 20% of eligible cases.

5.3 **Infusion of CTL**

5.3.1 Premedications: Patients will be premedicated with Benadryl up to 1mg/kg (max 50 mg) IV and Tylenol 10 mg/kg (max 650 mg) PO.

5.3.2 Cell Administration: Adeno-specific T cells will be thawed and given by intravenous injection initially from day 30 post transplant.

5.3.3 Location of treatment: All treatments will be as part of the Cell and Gene Therapy Transplant program at Texas Children’s Hospital or The Methodist Hospital. Patients may receive CTLs in the BMT clinic if they are an outpatient.

5.3.4 Monitoring will be undertaken according to institutional standards for administration of blood products with the exception that the injection will be given by a physician or a BMT team physician’s assistant or nurse practitioner. Premedication in appropriate dosage for each patient will be prescribed as necessary.

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

5.3.6 Concomitant medications: ideally, patients should not receive antiviral agents for adenovirus for at least 6 weeks post administration of CTLs (for purpose of evaluation), however, patients who need to be treated for potentially life-threatening viral infections such as CMV or HSV, will receive appropriate antiviral therapy.

5.3.7 If any patient develops clinical evidence of disseminated Adenoviral disease (enteritis, pneumonitis), treatment with Cidofovir will be initiated.
6.0 PATIENT EVALUATION

6.1 History and Physical Exam

A complete history and physical examination is necessary prior to administration of CTL.

6.2 Other Studies

6.2.1 The following investigations will be obtained pre-infusion, weekly until 60 days after the infusion, then at 3, 6, 9 and 12 months post infusion: CBC and diff, BUN, creatinine, bilirubin, SGOT, SGPT, alkaline phosphatase, LDH, Na, K, Cl, CO₂, albumin, total protein.

The following investigations will be obtained preinfusion, 24 hours post infusion (optional depending on patient preference), then on day 3 or 4 (optional depending on patient availability/preference) and at 1, 2, 4, 6 and 8 weeks post infusion. Follow up is then at 3, 6, 9, and 12 months post infusion: Peripheral blood in preservative free heparin (20-40ml) and in acid citrate dextrose (ACD) anticoagulant (3ml) will be obtained. This blood will be used for phenotyping analysis and analysis of specificity of CTL response using CD8 HLA-peptide tetramer analysis and immune function assays including ELISPOT and cytotoxicity assays. These studies will be done on patients on whom the appropriate reagents are available. If the patient has an additional infusion, the tests will also be obtained preinfusion, 24 hours post infusion (optional depending on patient preference), 3-4 days post infusion (optional depending on patient availability/preference), and at 1, 2, 4, 6 and 8 weeks post infusion. Patients will be monitored for 3 months for toxicity. Long term follow up for gene transfer (adenoviral vector used ex vivo) will then continue every 3 months until 12 months after the last infusion and after that they will be followed for long term outcome per transplant program guidelines.

6.2.2 Blood, +/- urine and/or stool (depending on clinical suspicion for adenovirus infection in these sites) will be screened for adenovirus by PCR assays on a weekly basis until 30 days after the infusion, then every second week for 2 months. If the patient has an additional infusion for Adenoviral infection, Adenovirus PCR assays will be monitored weekly until 30 days after the infusion, then every second week for 2 months. After that, monitoring will be done as clinically indicated.
6.2.3 If a patient’s hemoglobin is less than 8.0g/dl at any of the evaluation times, the amount of blood drawn for the evaluation will be reduced and may be obtained over more than one venipuncture, if necessary.

7.0 EVALUATION DURING STUDY

7.1 Follow-up interval

7.1.1 Patients shall be evaluated (seen in clinic, in the hospital or contacted by the research nurse) weekly for the first 60 days after the first CTL infusion, then at 3, 6, 9 and 12 months. After that they will be followed for long term outcome per transplant program guidelines. Additional visits will be obtained as clinically indicated or if the patient is having more than 1 infusion. Patients will be monitored for at least 30 days for dose escalation (as described in section 5.1) but for 3 months to assess late toxicity.

7.2 Early Termination of Study and Modifications

7.2.1 Potential Toxicities:
Graft versus host disease (GVHD): The risk that adoptively transferred Adeno-specific CTL will cause GVHD is very low. Any CTL cell lines with cytotoxic activity against patient-derived lymphoblasts or fibroblasts are excluded from patient use. Other toxicities: Should unanticipated toxicities arise (e.g. severe local reactions or hepatorenal damage) they, too, will be graded by NCI criteria.

7.2.2 As indicated below (section 8.0), a minimum of 12 patients will be enrolled, with 6 patients entered at the current MTD. Depending on patient availability, at most 18 patients will be enrolled into the study.

7.2.3 Therapy for an individual patient can be terminated if a dose-limiting toxicity has been observed. For purpose of this study, DLT will be defined as: development of Grade III-IV GVHD or NCI grade III-IV events in any organ system that may be possibly attributable to the study drug. Toxicity will be monitored for the
first 30 days after the last CTL infusion for dose escalation purposes and for a total of 3 months.

7.2.4 If the patient/parent desires to withdraw from the study or if the physician feels that it is in the best interest of the patient, treatment will be discontinued.

7.2.5 If grade 3 or 4 toxicity is encountered in these studies, the Institutional Review Board will be notified and dosage modifications made before admission of additional patients to confirm the maximum tolerated dose.

7.2.6 Although response is not the primary endpoint of this trial, patients with measurable Adenoviral infection will be assessed for ability of CTL to reduce Adenoviral load as determined by culture or PCR positivity for adenovirus in peripheral blood, urine, or stool.

7.3 Toxicity Grading
The criteria listed in the NCI Common Toxicity Criteria Scale will be used in grading toxicity. (Version 2.0 located at http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf). GVHD will be graded by the method of Przepiorka et al (see Appendix I). For consistency, toxicity for all patients will be quantitated using criteria listed in the NCI Common Toxicity Criteria Scale.

7.4 Evaluation Period
A 30 day period post CTL infusion will constitute the time for clinical safety monitoring for dose escalation as described in section 5.1, but patients will be monitored for clinical safety monitoring for a total of THREE months for long term toxicity.

8.0 STATISTICAL CONSIDERATIONS

8.1 This Phase I dose-escalation trial is designed to evaluate the safety of donor-derived adenovirus-specific cytotoxic T lymphocytes (CTLs) in patients at risk of developing adenovirus infection after allogeneic stem cell transplant. Dose-limiting toxicity is defined as development of Grade III-IV GVHD or NCI grade III-IV events in any organ system that can be attributed to the treatment (section 7.2.3.) Dose escalation is guided by the
modified continual reassessment method (mCRM) in order to determine the maximum tolerate dose (MTD) of adenovirus-specific CTL. As described in section 7.2.1, the risk that adoptively transferred Adeno-specific CTL will cause GVHD is very low. Thus, the target probability of acceptable toxicity is estimated to be \( \leq 20\% \). MTD is defined as the dose which causes DLT in at most 20% of eligible cases. Based on our previous trials, we expect a shallow dose-toxicity curve. Four dose levels are being evaluated namely, \( 5 \times 10^6 \), \( 1.5 \times 10^7 \), \( 4.5 \times 10^7 \), and \( 1.35 \times 10^8 \) with prior probabilities of toxicity estimated at 3%, 5%, 10%, and 25%, respectively. In this trial, mCRM is implemented based on logistic model with a cohort of size 2\(^2\). To reduce the probability of treating patients at unacceptable toxic dose levels, we employ modifications to the original CRM\(^2\). Specifically, there will be more than one subject treated in each cohort, dose escalation is limited to no more than one dose level, and patient enrollment starts at the lowest dose level shown to be safe in previous studies of CTL post HSCT.

Two patients are allocated in each cohort and are followed for 30 days post CTL infusion for evaluation of DLTs. The trial continues until a minimum of 12 patients is treated with six patients accrued at the current MTD. Depending on patient availability, a maximum 18 patients will be accrued into this Phase I trial. The final MTD will be the dose with probability closest to the target toxicity rate at these termination points. We therefore expect to enroll between 12-18 patients into this trial.

We performed simulations with 10,000 replications to determine the operating characteristics of the proposed design and compared this with a standard 3+3 dose-escalation design. Our proposed design provides better estimates of the MTD based on a higher probability of declaring the appropriate dose level as the MTD, afforded smaller number of patients accrued at lower and more likely ineffective dose levels, and maintained a lower average total number of patients required for the trial. As indicated above, the first dose level used is lower than the dose used in previous studies of CTL post HSCT and we expect a shallow dose-toxicity curve over the range of doses we propose in our study. Therefore, we feel comfortable with slightly more accelerated dose-escalations without compromising patient safety. In fact, our simulations indicate that the average number of total toxicities will be about 1.7 in the modified CRM and 1.6 in the standard design.
DLTs that occur within 30 days after initial infusion will be factored into the CRM calculations to determine the recommended dose for the subsequent cohort. During the study, real-time monitoring of patient toxicity outcome will be performed in order to implement estimation of the dose-toxicity curve and determine dose level for the next patient cohort using one of the pre-specified dose levels.

8.2 Data Analysis

Safety and toxicity outcomes including DLTs, GVHD, and laboratory evaluations will be summarized by dose levels using descriptive statistics. Immunological parameters based on ELISPOT and cytotoxicity assays, and frequency of cells secreting γ-IFN will be summarized using descriptive statistics at each time point. Growth curves of immune response over time within a patient will be generated to visualize general patterns of immune response. Pairwise comparisons will be performed to compare changes of these immunological parameters from pre-infusion to each time point of post-infusion measurements using paired t-tests or Wilcoxon signed-ranks tests. Longitudinal analysis is employed to model repeatedly-measured immunologic parameters. This will allow us to model patterns of immune response per patient while allowing for varying intercepts and slopes for a patient. We will also include dose level as an independent variable in the model to account for the different dose levels received by the patients. The normality assumption will be assessed and transformations to achieve approximate normality will be carried out if necessary.

Viral load levels will be correlated with levels of CTLs and immunological parameters using correlation coefficients. Similar longitudinal modeling strategies will be employed to analyze the viral load based on quantitative real time PCR. These models will also be utilized to model persistence of CTLs as a function of viral load measurements over time and viral load levels as a function of immunological parameters. These modeling strategies will be considered exploratory in nature due to the limited patient numbers in this initial trial.

9.0 STUDY INTERPRETATION

9.1 Since this is a Phase I study, the main aim will be to collect information about the toxicity. As secondary endpoints, we will estimate the effective cell dose of Adeno-specific CTLs in preventing or treating Adenoviral infection/disease in this patient population, evaluate recovery of virus-specific immunity in patients after CTL infusion and its correlation
with protection from viral reactivation/disease. This will allow us to collect data on immunomodulatory and virological efficacy for a future Phase II study.

9.2 Response Criteria

9.2.1 Response

No evidence of Adenoviral infection as detected by adenoviral culture or PCR three months following CTL infusion or at least a 2-fold increase in the frequency of adenovirus-specific T cells detected in the blood either by multimer (pentamer or tetramer) or IFN\(\gamma\) ELISPOT assay. In patients who have Adenoviral infection and are treated with CTL, response will be determined from the reduction (at least 2-fold) in Adenoviral DNA positivity and/or an increase in frequency of Adeno-specific CTL precursors as measured by IFN\(\gamma\) ELISPOT assay and/or HLA peptide tetramer positive cells.

9.2.2 Non Response

Development of disseminated Adenoviral disease any time after CTL infusion: should Adenoviral disease be suspected, biopsies of appropriate sites such as the GI tract and liver biopsies or evaluation of BAL fluid or lung tissue will be obtained.

10.0 RECORDS TO BE KEPT

The CAGT data manager will maintain a database documenting the dates and doses of therapy as well as a flow sheet listing clinical chemistries and hematologic parameters. The clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

- Imaging reports
- Surgical summaries
- Autopsy summaries, where appropriate
- Informed consent documents

All required clinical evaluation records will be the responsibility of Dr. Bollard, who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of immunological efficacy will be the responsibility of Dr. Bollard.
11.0 REPORTING REQUIREMENTS

11.1 Register all patients with Cell and Gene Therapy Research Nurse.

11.2 Enter all patients by calling Dr Bollard. The following forms should be completed:
   Eligibility check list
   pre study form
   Adverse event form
   Flow sheets
   Response form
   Off study form
   Death form

11.3 Drug Toxicity and/or Adverse Reactions

11.3.1 Within one (1) working day, report by telephone to the Principal Investigator or research nurse serious adverse events as defined below:
   - **Associated with the use of the biologic**: There is a reasonable possibility that the experience may have been caused by the biologic.
   - **Disability**: A substantial disruption of a person’s ability to conduct normal (change from baseline at study entry) life functions.
   - **Life threatening adverse biologic experience**: Any adverse biologic experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred.
   - **Serious adverse biologic experience**: Any adverse biologic experience occurring at any dose that results in any of the following outcomes:
     - Death (all fatal events regardless of causality)
     - A life-threatening adverse biologic experience
     - Inpatient hospitalization or prolongation of existing hospitalization
     - A persistent or significant disability/incapacity
     - A congenital anomaly/birth defect
     - Any other medical event that, in appropriate medical judgment, may require medical or surgical intervention to prevent one of the outcomes listed above.
• **Unexpected adverse drug/biologic experience**: Any adverse biologic experience, the specificity or severity of which is not consistent with the current investigator brochure, or, not consistent with the risk information described in the general investigational plan (protocol).

11.3.2 All serious adverse events as outlined in section 11.3.1 should also be reported within three (3) working days, in **writing**, to the Principal Investigator. An appropriate adverse event form should be used.

11.3.3 Individual Institutions are responsible for reporting serious adverse events occurring in the first 3 months to their institutional review board (IRB) according to each board’s required timeframe.

11.3.4 The Sponsor (IND holder) will be responsible for submitting all reportable adverse events to the appropriate regulatory agencies (e.g. FDA/RAC/NCI as applicable) as per each agencies individual reporting requirements and within each agencies required timeframe. This reporting will continue for 1 years.

11.3.5 Data on **all** adverse experiences/toxicities, regardless of seriousness, must be collected for documentation purposes only. AEs should be followed for 3 months after the last dosing of study drug/biologic.

11.3.6 Non-serious adverse events will be reported to the appropriate regulatory agencies (e.g. IRB/FDA/RAC/NCI as applicable) at the time of the annual report if required by that agency.

11.3.7 Reporting will continue for 1 years for events related to the gene transfer. Data on AEs must be collected for documentation purposes only for 3 months after CTL.

12.0 **INFORMED CONSENT**
All patients and/or their legal guardian must sign a document of informed consent consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be
informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted for treatment without such a document signed by him or his legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines.

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

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

Reference List


(13) Myers GD, Krance RA, Weiss H et al. Adenovirus infection rates in pediatric recipients of alternate donor allogeneic bone marrow transplants receiving either antithymocyte globulin (ATG) or alemtuzumab (Campath). Bone Marrow Transplant. 2005.


15.0 APPENDIX II

GVHD STAGES AND GRADES

For skin:  Stage  Skin Involvement
0  0
1  greater than 0, less than 25%
2  greater than or equal to 25%, less than or equal to 50%
3  greater than 50%
4  greater than 50% with blisters

For gut:  Stage  Stool Volume
0  less than 7 cc/kg
1  greater than or equal to 7 cc/kg, less than 14 cc/kg
2  greater than or equal to 14 cc/kg, less than 21 cc/kg
3  greater than or equal to 21 cc/kg, less than 28 cc/kg
4  greater than or equal to 28 cc/kg

For liver:  Stage  Bilirubin (mg/dL)
0  less than 2
1  greater than or equal to 2, less than 3
2  greater than or equal to 3, less than 6
3  greater than or equal to 6, less than 15
4  greater than or equal to 15

Overall:  Grade  Organ Stage
0  0
1  skin = 1 or 2
2  skin = 3, or skin less than or equal to 3 and gut or liver equal to 1
3  skin greater than or equal to 3 and gut or liver equal to 2 or 3
4  skin, gut or liver equal to 4