ADMINISTRATION OF DONOR-DERIVED MULTI-TUMOR-ASSOCIATED ANTIGEN (TAA)-SPECIFIC T CELLS TO PATIENTS WITH AML OR MDS (ADSPAM)

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ADSPAM CHECKLIST FOR TREATMENT ELIGIBILITY

PATIENT ID _____ **PATIENT NAME** _____

YES	NO	VALUE/DATE	
Any "NO" answers will make a patient ineligible for study participation.			
			Group A: Adjuvant therapy for AML/MDS patients at least 30 days post-allogeneic HSCT; or
			Group B: AML/MDS patients at least 30 days post-allogeneic HSCT with minimal residual or refractory/relapsed disease. Residual disease at the time of transplant or post transplant relapse is defined as PCR positivity, specific cytogenetic abnormalities, an abnormal population on flow cytometry or increased blasts on bone marrow biopsy,in the peripheral blood, or any other extramedullary sites. MRD will be defined as detection in blood, bone marrow, or other tissues any of the following: • Detection of a pre-transplant leukemia-specific marker (such as t(8;21); inv 16; t (15;17), t(9;22) or t(4;11)) on a post-transplant evaluation • Expression of a leukemia associated antigen known to be a marker for residual disease including WT1 • A leukemia-specific phenotype (e.g. expression of markers including CD13 and/or CD33 and/or CD117 and/or HLA-DR+) post-transplant at a level of ≥ 0.01% • Mixed donor chimerism (> 20%)
			Life expectancy ≥ 6 weeks
			Undergoing Stem Cell Transplant at CAGT
			Karnofsky/Lansky score of ≥ 50
			Patient or parent/guardian capable of providing informed consent
			Bilirubin ≤ 2 x upper limit of normal
			AST ≤ 3 x upper limit of normal
			Serum creatinine ≤ 2 x upper limit of normal
			Hgb ≥ 7.0 g/dL (can be transfused)
			Pulse oximetry of > 90% on room air
			Sexually active patients must be willing to utilize one of the more effective birth control methods for 6 months after the T cell infusion
			Available donor-derived multi-TAA specific T cell line
			No other investigational anti-neoplastic therapy for one month prior to entry in this study
Any "YES" answers will make a patient ineligible for study participation.			
			Less than 30 days from date of allogeneic stem cell transplant
			Severe intercurrent infection
			Evidence of GVHD > grade II
			Pregnant or lactating
			Patients receiving a Donor Lymphocyte Infusion within 4 weeks of planned T cell infusion
			Has received Alemtuzumab or antithymocyte globulin (ATG) within the past 28 days
			Currently taking corticosteroids (> 0.5 mg/kg/day prednisone or equivalent)

Signature of MD _____ Date _____

1.0 OBJECTIVES

1.1 Primary Objective - To determine the safety of an intravenous injection of donor-derived multi-tumor associated antigen (multiTAA)-specific T cells, administered as prophylaxis or treatment of AML or MDS post allogeneic hematopoietic stem cell transplant (HSCT).

1.2 Exploratory Objective 1 - To obtain information on the expansion, persistence and anti-tumor effects of the adoptively transferred donor-derived multiTAA-specifc T cells in patients with AML or MDS.

1.3 Exploratory Objective 2 - To determine whether multiTAA-specific T cells can increase the spectrum of epitopes/antigens targeted by endogenous T cells (epitope spreading).

2.0 BACKGROUND AND RATIONALE

2.1 Introduction

Acute myeloid leukemia (AML) and myelodysplatic syndromes (MDS) have been proven to be sensitive to immune-based intervention. Indeed, the graft versus leukemia (GVL) effect mediated by adoptively transferred unmanipulated donor T cells following allogeneic hematopoietic stem cell transplant (HSCT) is one of the most striking examples illustrating the benefit of harnessing the immune system. Unfortunately, these benefits must be weighed against the coincident risk of inducing acute or chronic graft versus host disease (GVHD). In the current study we propose enhancing the GVL effect while simultaneously mitigating the risk of inducing GVHD by selectively amplifying donor-derived tumor-targeted precursors ex vivo using repetitive rounds of stimulation with antigen presenting cells (APCs) expressing a range of antigens that are selectively overexpressed on malignant cells.

2.2 Acute Myeloid Leukemia (AML)

AML is a malignant neoplasm of myeloid lineage cells arising within the bone marrow and outgrowing normal hematopoietic elements. Although AML can present at all ages, it has a bimodal age distribution, with one peak within the first 10 years of life and a much larger peak beyond age 60. In 2014 there will be an estimated 52,380 new cases of leukemia in the United States [1].

Adult patients with AML are stratified based on cytogenetics and molecular features into low, intermediate and high risk disease groups to identify those who would benefit from allogeneic HSCT in first remission. The standard first-line treatment approach for the past 4 decades regardless of risk stratification has been combination chemotherapy using cytarabine and an anthracycline, which has produced initial complete remission (CR) rates in 50-80% of patients. However, approximately half of these patients eventually relapse. Eligible patients subsequently proceed to allogeneic HSCT, but disease relapse is frequent (~60%) and is a major cause of death in these patients[2]. Overall, patients who are not cured with front- or second-line therapy have an estimated median survival of < 1 year[2], highlighting the need for novel therapies. Pediatric patients are similarly risk stratified with disease response as well as molecular features

and cytogenetics being critical to assessment of prognosis. With improved therapy as well as supportive care, pediatric patients have ~70% chance of survival at 5 years from diagnosis. However, patients with refractory or relapsed disease (~35%) have a very poor prognosis. This subset is generally offered re-induction chemotherapy followed by allogeneic HSCT. Outcomes are improved with disease remission at the time of transplant, but overall survival in this group remains < 30%[3].

2.3 Myelodysplastic Syndromes (MDS)

MDS represent a heterogeneous group of clonal stem cell disorders resulting in refractory anemia associated with dysplastic morphologic bone marrow features. The estimated incidence of MDS is 4.6 per 100,000 but the majority of cases are diagnosed after the 7th decade of life (median age of diagnosis – 76 years) with a rate of nearly 50 per 100,000 after age 70[4]. Prognosis is directly related to the number of bone marrow blast cells, cytogenetic abnormalities, and to the number of affected hematopoietic cell lineages as measured by blood counts. There is considerable variability in the clinical course of patients with MDS. Patients who develop MDS as a consequence of prior chemotherapy or radiation, known as treatment-related MDS or t-MDS typically have an aggressive course and a rapid progression to leukemia, whereas in de novo cases, the disease can follow an indolent course or, with the acquisition of additional genetic mutations, can eventually progress to AML.

In adults, low risk patients with indolent disease have an expected median survival of 5.7 years and are less likely to die because of progression to leukemia. In contrast, patients with high-risk disease can progress to leukemia within 0.2 years with a median overall survival of 0.4 years[5]. Most patients with symptomatic MDS are treated with hypomethylating agents (e.g. azacytidine) or immunomodulators like lenalidomide[6]. While hypomethylating agents have been shown to induce partial and complete responses in 15-30% of patients, the benefits are usually sustained only short-term, followed by disease progression[7]. Without an allogeneic HSCT, MDS is considered an incurable disease. Pediatric patients rarely present with isolated anemia but more commonly are diagnosed with MDS following examination of clinical symptoms related to at least bilineage cytopenia. The disease is rare in the pediatric patient, but an important diagnosis secondary to propensity for evolution to frank leukemia. Pediatric MDS can be associated with inherited bone marrow failure predisposition syndromes and, as in adults, may be secondary to disease-targeted therapy. The only curative treatment is HSCT.

2.4 Targeting Malignancies with Adoptively Transferred T Cells

2.4.1 Targeting virus-associated malignancies using T cell therapy

The adoptive transfer of antigen-specific T cells has been associated with dramatic clinical benefit when used to prevent and treat a range of viral infections[8] and some virus-associated tumors[9]. For example, adoptively transferred donor-derived Epstein-Barr virus (EBV)-specific T cells have produced complete and durable remissions in > 70% of patients with EBV-associated post-transplant lymphoproliferative disease (PTLD) with minimal toxicity[10]. Adoptive T cell transfer has also produced clinical responses outside of the transplant setting in patients with EBV-associated lymphomas and nasopharyngeal carcinoma[9,11]. When T cell lines targeting the tumor-expressed EBV antigens LMP1 and LMP2 were infused to a total of 50 patients with EBV type II or III latency lymphomas 28 of 29 patients who received the cells as adjuvant therapy remained in CR, while in the treatment arm 11 of the 21 patients (52%) achieved a CR and 2 patients achieved a PR. Importantly, complete responses were seen even in patients with limited apparent in vivo expansion of LMP-directed T cells. Further, clinical benefit was associated with epitope spreading and the emergence of de novo cellular

immune responses directed against non-viral tumor-expressed antigens including the cancer testis antigens (CTAs) PRAME and MAGE-A4, as well as Survivin, a "universal" tumor antigen expressed in most human neoplasms[12].

2.4.2 Considerations when developing an immunotherapy for non-viral tumors

To develop an immunotherapeutic strategy to treat patients with EBV-negative lymphomas we developed an alternate T cell based approach targeting non-viral tumor associated antigens (TAAs). From an immunotherapeutic perspective the model tumor antigen to target should be one that is exclusively and universally expressed on tumor cells in order to limit collateral damage, and ideally should be essential for the maintenance of the oncogenic phenotype of the tumor. However, the majority of antigens do not meet these criteria since they are not neo-antigens uniquely present in malignant cells but rather antigens that are also expressed in normal cells and against which peripheral blood T cells are tolerized or deleted. Tumor-specific antigens have nonetheless been identified, and these can be classified into 4 groups;

(i) Unique antigens (e.g. MUM1) result from single mutations that are tumor and patient specific and therefore are only expressed in neoplastic cells. They are often considered ideal for immunotherapy since tumor cells can be specifically targeted without destroying nearby normal tissue, and they may also be relatively strong antigens[13]. However, because they are also usually patient-specific, the identification of the mutated gene and then the generation of an individualized T cell product targeting the identified antigen is highly labor and cost intensive[13,14].

(ii) The shared lineage-restricted antigens, expressed on tumor cells as well as their normal tissue of origin, such as the melanoma associated antigens MART, gp100 or Melan-A. These antigens are also strongly immunostimulatory, equivalent almost to "weak" viral antigens, enabling the efficient and relatively simple generation and expansion of tumor-specific T cells from healthy donors and patients with minimal in vitro manipulation[14]. However, T cell mediated destruction of normal melanocytes, for example, has resulted in vitiligo as well as ocular and systemic autoimmunity in patients treated with melanoma-specific T cells or tumor infiltrating lymphocytes (TILs)[14].

(iii) Shared tumor-specific TAA (e.g. the cancer testis antigens [CTA] - MAGE, BAGE, GAGE, NY-ESO-1, SSX, PRAME) are expressed in multiple tumors but not in healthy organs, with the exception of germ line tissues that are immune privileged and therefore not susceptible to T cell attack. Most CTAs have heterogeneous expression in cancer tissues and are frequently expressed in high-grade or late tumor stages, with expression often correlating with a worse prognosis. Furthermore, tumors expressing one CTA are also often found to express multiple CTAs, and several have been found to be targets of spontaneous humoral or cell-mediated immune responses[15]. Thus, CTAs are particularly attractive as targets for tumor immunotherapy since reactive T cells can be produced on a large scale to provide broad-spectrum protection against a variety of tumors. CTAs have been targeted in both vaccine and T cell therapy protocols, with evidence of clinical efficacy[16,17,18,19,20,21].

(iv) The last group are antigens that are overexpressed in many different tumors but expressed at low levels in healthy tissue (eg. hTERT, CEA and Survivin). T cells targeted to these antigens carry the risk of inducing collateral damage to normal tissues co-

expressing the antigen (e.g. CEA and normal biliary epithelium), and there are limited clinical data available regarding the safety of targeting these antigens in vivo. However, Survivin- and CEA-specific T cells have been isolated from the peripheral blood of patients who have cleared their tumors, and increases in Survivin-specific T cells in patients receiving oncolytic viruses have been reported, suggesting that they can have efficacy without toxicity in patients[22,23,24,25,26,27].

2.4.3 Targeting EBV-negative lymphomas using adoptive T cell transfer

With the goal of developing an adoptive immunotherapeutic approach to treat patients with EBV-negative lymphomas we developed a protocol for the in vitro generation of T-cell lines targeting non-viral TAAs. Since the expression of most tumor antigens is heterogeneous among tumors from different patients and can even vary among metastases obtained from different sites from the same patient we decided to target a combination of TAAs frequently expressed by Hodgkin lymphoma (HL) and Non-Hodgkin lymphoma (NHL), and against which we had previously detected immune responses in our EBV-directed studies[12]. Thus, we focused on Survivin as well as the CTAs PRAME, MAGE-A4, NY-ESO-1 and SSX2[15]. We generated clinical grade peptide mixtures (pepmixes) consisting of 15mer peptides overlapping by 11 amino acids spanning the entire sequence of each of these antigens to generate T-cell lines that recognize multiple TAAs (multiTAA-specific T cells). Briefly, T cells were activated using pepmix-loaded dendritic cells (DCs) as antigen presenting cells (APCs) and expanded in a cytokine cocktail that included IL7, IL12, IL15, and IL6[28,29].

To date we have infused a total of 10 patients with these multiTAA-specific T cells (NCT01333046) without infusion-related toxicities. One patient with relapsed disease initially had disease stabilization followed by progression; 6 patients received the cells as adjuvant therapy and all but one (with prior history of multiply relapsed disease) remain in remission while 3 patients with active disease at the time of receiving cells all achieved CRs. Interestingly, in responders we detected evidence of epitope spreading and the emergence of de novo cellular immune responses directed against other non-targeted tumor-expressed antigens including MAGE-A2B, MAGE-C1 and AFP.

2.4.4 Applying immunotherapeutic approaches to treat AML and MDS

Based on the promising clinical responses that have been achieved using ex vivo expanded T cells to target lymphoma we now want to apply a similar strategy to target AML and MDS.

2.4.5 Targeting TAAs in AML and MDS

AML blasts express several T cell immunogenic tumor antigens that fall into two categories: (i) minor histocompatibility antigens (mHAgs), and (ii) TAAs overexpressed by the leukemic cells with limited expression on normal cells.

2.4.5.1 Minor histocompatibility antigens

mHAgs are HLA-binding peptides derived from endogenous proteins in cells of the stem cell transplant recipient that differ from those of the donor due to genetic polymorphisms and thus represent a unique class of antigens that can be targeted only after allogeneic HSCT to promote both GVL and GVHD effects in vivo[30]. Much of the current research in this area has focused on identifying and selectively targeting mHAgs expressed exclusively on malignant cells. Warren and colleagues recently evaluated the safety of adoptively transferring donor-derived CD8+ T cell clones recognizing mHAgs preferentially expressed on hematopoietic cells to patients with relapse of acute leukemia after myeloablative allogeneic HSCT. The highest cell doses

administered to each patient ranged from 2.25-6.6 x 10^9 cells. Pulmonary toxicity was seen in three of the seven treated patients, and was severe in one, and correlated with the level of expression of the mHAg-encoding genes in lung tissue. However, the administration of steroids coincided with a rapid reversal in pulmonary symptoms. Thus the associated toxicity could be rapidly and effectively controlled[31].

2.4.5.2 Tumor-associated antigens

In both the preclinical and clinical setting TAAs that are overexpressed on malignant cells (e.g. Wilms Tumor-1, WT1; Survivin; and the CTAs PRAME and NY-ESO1) have been targeted using a range of immunotherapeutic strategies including antibody-based therapies, peptide vaccines and adoptive T cell transfer approaches. **Table 1** shows the frequency of expression of select TAAs in AML and MDS based on published reports.

Table 1: Frequency of TAAs expressed in AML and MDS[32,33,34]

	WT1	SURVIVIN	PRAME	NY-ESO-1
AML/MDS	70-90%	72-100%	30-60%	0-7%

WT1: WT1 is a nuclear zinc finger transcription factor that is essential during embryonic development of the urogenital system. Post-natally, WT1 expression in healthy tissues is limited to the ovary, testis, podocytes of the kidney and the mesothelial linings of the peritoneum and pleura. Additionally, WT1 is expressed at low levels in hematopoietic progenitor cells where it normally acts to induce quiescence of $CD34^+$ Lin^- cells and promote differentiation of precursors at later stages of development[35]. In contrast, WT1 is highly expressed in several solid tumors, and >70% of AMLs affecting children and adults; high levels of WT1 expression are associated with poor prognosis[36]. WT1 is also aberrantly expressed in chronic myeloid leukemia and in advanced forms of myelodysplasia[37]. In leukemic blasts, the balance of WT1 isoforms expressed appears to promote proliferation and resistance to apoptosis while inhibition of WT1 expression (eg using shRNA) reverses these effects, thereby eliminating leukemic cells with clonogenic potential[38,39].

The clinical relevance of targeting this tumor-expressed antigen using an immunotherapeutic approach is evidenced by the fact that disease control or remission in several vaccine studies has been associated with the induction of WT1-specific T cells, and an increased frequency of circulating WT1-specific T cells post HSCT has been associated with sustained disease remission[40,41,42]. In a seminal phase I study, Oka and colleagues studied the effects of administering an HLA-A24-restricted WT1-peptide vaccine to 11 patients with AML in morphologic remission but with minimal residual disease. Nine of 11 evaluable patients had a detectable increase in the frequency of WT1-specific T cells post-vaccination, which correlated with a clinical response and notably 2 treated patients remained in CR >8 years[43]. Similarly, Keilholz and colleagues conducted a phase II trial with an HLA-A0201 restricted WT1 peptide vaccine in 17 patients with active AML and 2 patients with MDS. Overall, 14 of 17 AML patients and both patients with MDS had a clinical response to therapy which included one CR and four PRs (>50% reduction in blasts) with no adverse events ≥III grade and no myelosuppression[44]. To induce a broader spectrum of T-cell responses in vivo

Tendeloo and colleagues vaccinated 10 AML patients with residual disease post chemotherapy using mRNA-transfected DCs and reported 5 complete molecular responses, as measured by flow and WT1 levels, that were sustained for at least 2 years in 3 patients[45]. WT1 has also been targeted using adoptively transferred T cells. For example Greenberg and colleagues generated donor-derived HLA-A2 peptide-directed CD8+ T cells using peptide-loaded DCs as APCs. These were administered to AML or ALL patients post allogeneic HSCT, 4 of whom had active disease at the time of infusion. The infusions proved safe with toxicity limited to tolerable transient infusion site reactions. Furthermore, the infused cells produced clinical benefit. Indeed, in the 4 patients with active disease the transferred cells exhibited direct anti-leukemic activity in 3 resulting in two CRs and one PR and these clinical responses correlated with the detection of tetramer positive WT1 specific T cells in the periphery[46]. Finally, O'Reilly and colleagues have an ongoing clinical trial using donor-derived WT1-specific T cells activated using DCs loaded with an overlapping peptide library (15mers overlapping by 11 amino acids) spanning the entire sequence of WT1. These cells are being infused to patients with persistent minimal residual disease or recurrence of WT1+ AML, ALL, or MDS following allogeneic HSCT (NCT00620633). In preliminary reports WT1 T cell infusions at the lowest dose levels were reported to be safe and well tolerated (ASBMT 2014, abstract # 24 and 48).

Survivin: Survivin is a member of the inhibitor of apoptosis protein family and is overexpressed in the majority of tumors including esophageal, lung, ovarian, breast, and colorectal cancer as well as most hematologic malignancies[47]. Elevated Survivin expression is commonly associated with resistance to chemotherapy, enhanced proliferative capacity and, in the case of AML, it is an independent unfavorable prognostic factor[32].

Survivin has also been targeted clinically. For example, Andersen and colleagues developed an HLA-A0201 Survivin peptide vaccine, which was administered to 5 patients with advanced melanoma. All had robust increases in Survivin-specific T cells resulting in disease stabilization for the 3 month duration of the vaccinations in 4 individuals without major toxicities[48]. Similarly, a patient with metastatic pancreatic cancer had a CR of liver metastasis following the administration of an HLA-A2 Survivin peptide vaccine[49]. With regards to hematologic malignancies, Rapaport and colleagues used an HLA-A2 Survivin/hTERT peptide vaccine in 28 autologous HSCT recipients with myeloma. The vaccine was well tolerated and resulted in increased numbers of Survivin-specific T cells in 10 individuals, as measured by tetramer analysis[50]. Finally there are several ongoing Survivin-targeted phase I/II clinical trials including in patients with melanoma (NCT01543464), ovarian cancer (NCT01456065), leukemia (ALL and AML, NCT00664677) and glioma (NCT01250470), though the results of these studies are not yet publically available.

PRAME: The CTA preferentially expressed antigen of melanoma (PRAME), was initially identified as a tumor antigen in melanoma but has since been found to be overexpressed in many hematologic malignancies and solid tumors, while its expression is low or absent in normal tissues[51]. PRAME may significantly contribute to maintaining the tumor phenotype, because its expression can strongly inhibit cell differentiation induced by the retinoic acid receptor-α ligand all-*trans* retinoic acid, a crucial pathway for the proliferation and differentiation of both normal and malignant

hematopoietic cells. Indeed, it has recently been demonstrated that PRAME overexpression contributes to leukemogenesis by inhibiting myeloid differentiation through blockage of the retinoic acid receptor-α–signaling pathway[52].

Anti-PRAME immunity in AML was first demonstrated by the presence of PRAME-specific T cells in samples from patients in remission from AML and MDS post allogeneic HSCT. In these patients relapses could be predicted by rising PRAME transcript levels and declining PRAME-specific T-cell numbers[53,54]. Rezvani and colleagues corroborated and further extended these findings and showed that naturally occurring PRAME-specific CD8+ T cells directed against a spectrum of immunogenic epitopes were detected at much higher frequencies in patients with PRAME-expressing AML, ALL and CML cells compared with individuals whose leukemic cells had low/absent PRAME expression[55]. Several groups including ours have confirmed the immunogenicity of PRAME. Indeed, we were able to efficiently activity and expand PRAME-directed T cells using an overlapping peptide library spanning the entire protein sequence and demonstrated that these in vitro-expanded T cells were able to kill partially HLA-matched primary cell lines as well as leukemia progenitors in vitro[16,17,28,56]. PRAME has been safely targeted in patients with advanced solid malignancies. For example, in a phase I vaccine study Weber et al administered a PRAME HLA-A2 restricted peptide and a recombinant plasmid encoding fragments of PRAME to 24 patients with advanced solid tumors and induced reactive T cells in 15[57]. Further, our group has an ongoing clinical trial targeting PRAME using adoptively transferred T cells in patients with relapsed/refractory HL and NHL (NCT01333046).

NY-ESO1: NY-ESO1 is a highly immunogenic CTA aberrantly expressed in a variety of malignancies including melanoma, lung cancer, sarcoma, multiple myeloma and leukemia while normal tissue expression is limited to germ line tissues which lack MHC molecules. It was first discovered as an immunogenic CTA based on the detection of specific antibodies in the sera of patients with esophageal carcinoma[15]. Later, it was also shown to induce CD8+ and CD4+ T-cell responses in patients with NY-ESO-1 bearing tumors[18]. In a striking observation, Wolchock and colleagues noted a clear association between clinical benefit and the detection of NY-ESO-1-specific antibodies in melanoma patients treated with ipilimumab, suggesting its immunotherapeutic importance[58].

To specifically target NY-ESO-1 Dhodapkar and colleagues developed a recombinant NY-ESO-1 protein/adjuvant vaccine and all 46 vaccinated patients with resected NY-ESO-1 positive tumors developed an immune response manifested by strong local site delayed hypersensitivity reactions followed by the activation of CD4+ and/or CD8+ NY-ESO-1-reactive T cells[59]. In a separate clinical trial, full length NY-ESO-1 antigen combined with recombinant fowlpox or vaccinia virus was administered as a vaccine to patients with a spectrum of NY-ESO-1 bearing tumors. Again, the vaccines were well tolerated, and all patients developed NY-ESO-1-specific antibody and/or CD4 and CD8 T cell responses directed against a broad range of NY-ESO-1 epitopes. Clinically, 9 of 16 evaluable patients with advanced metastatic cancers had objective responses including one CR in a melanoma patient[60].

To enhance the immunogenicity, Bender et al administered five weekly (as opposed to once weekly) doses of 100 µg of an HLA-A2 restricted NY-ESO-1 peptide to 20 patients with advanced NY-ESO-1 tumors. The only adverse effects reported were transient grade I and II mainly skin reactions. Eleven patients had an increase in the frequency of specific T cells post-vaccination, which appeared to correlate with a better overall survival[61]. This antigen has also been targeted using adoptively transferred T cells. Hunder et al reported a durable CR achieved in a patient with advanced melanoma who was infused with autologous peptide-directed CD4+ T-cell clone. Except for an elevated temperature to 38.1C for 4 hours post-infusion no adverse events were reported despite detection of the infused cells through PCR for the TCR gene for 80 days[62]. The adoptive transfer of T cells engineered with specificity for NY-ESO-1 via transgenic expression of a TCR directed against a A2 epitope was also found to be safe in 17 patients with advanced sarcoma or melanoma and resulted in tumor regression in 4 of 6 sarcoma and 5 of 11 melanoma patients[63].

Though NY-ESO-1 is expressed infrequently in AML (up to 7%) conventional therapeutic agents including hypomethylating agents can induce antigen expression on blasts[33]. Indeed, Goodyear et al. demonstrated the induction of NY-ESO-1-specific CD8+ T cells after treatment with the hypomethylating agent 5-azacytidine and decitabine resulting in development of major clinical responses in 8 patients (4 CRs and 4 PRs) who had active relapsed AML prior to study[64].

2.5 Choosing the Optimal TAA Targets

The CTAs NY-ESO-1 and PRAME are expressed to varying degrees in leukemic stem cells and blasts (**Table 1**). In addition, WT1 is expressed in 70-90% of AML/MDS leukemic cells, while Survivin is ubiquitously expressed on malignant cells. The expression of these tumor antigens in AML and MDS and their evident immunogenicity make them potential targets for T cell therapy[32,33,34]. Although most tumor cells express one or more of these antigens, there may be differences from patient to patient and differences also between individual tumor cells in levels of antigen expression. Thus, in order to provide potential clinical benefit to the majority of patients we propose generating T cell lines with simultaneous activity against NY-ESO-1, PRAME, Survivin, and WT1 and testing the safety of these cells in a dose escalation study. Additionally, we will assess the frequency with which these antigens are expressed on the tumor cells, though we will not use antigen expression as an eligibility criterion for this study.

As a means of stimulating T cells we will use clinical grade peptide mixtures (pepmixes) that consist of 15mer peptides overlapping by 11 amino acids spanning the entire sequence of NY-ESO-1, Survivin, WT1 and PRAME. These peptide libraries encompass all possible HLA class I epitopes and the majority of HLA class II epitopes of each protein. Using this approach we expect to generate T cell lines with specificity for up to four antigens as has been our experience in pre-clinical validation studies[29]. Since the individual peptides are 15 amino acids in length we anticipate activating both CD4+ and CD8+ T cells.

2.6 Risks of Administering Tumor-Specific T Cells

It is possible that the infusion of T cells targeting self antigens expressed in normal tissue may induce an inflammatory response post-infusion, as reported by Warren and colleagues[31]. These investigators evaluated the safety of adoptively transferring donor-derived CD8+ T-cell clones recognizing mHAgs to patients with relapse of acute leukemia after myeloablative allogeneic

HSCT. The highest doses administered to each patient ranged from 2.25-6.6 x 10^9 cells. Pulmonary toxicity was seen in three of the seven treated patients, and was severe in one, and correlated with the level of expression of the mHAg-encoding genes in lung tissue. However, the administration of steroids coincided with a rapid reversal in pulmonary symptoms. Thus, the associated toxicity could be rapidly and effectively controlled. We do not anticipate such problems with the current protocol since;

(i) Our proposed infused cell doses are the same as those being used in our current lymphoma study (which has proven safe to date) and these doses are 2-3 logs lower than the Warren study and a log lower than our previous LMP (EBV virus-specific) T-cell study in which we saw no toxicity.

(ii) The T cells are polyclonal, directed against multiple rather than single epitopes/antigens.

(iii) NY-ESO-1 and PRAME are not expressed on normal tissue except germ line tissues.

(iv) WT1 is only present at low levels in nephrons and hematopoietic cells. Oka et al observed myelosuppression after administration of WT1 directed vaccine in 2 MDS patients[43]. However this was postulated to be an anti-tumor effect because there was a reduction in WT1 mRNA levels. Regardless those patients were removed from the study. Since then there have been 2 WT1 vaccine trials and 2 WT1-specific T-cell adoptive transfer trials with nearly 10 patients with MDS without the development of myelosuppression and there have been no reports of nephritis following immunotherapeutic approaches targeting WT1[44,65].

(v) Survivin is expressed only at low levels in restricted normal cell types such as thymocytes, T cells, basal colonic epithelial cells and CD34+ bone marrow derived stem cells. High Survivin expression has been reported in tumors of lung, breast, colon, stomach, esophagus, pancreas, liver, uterus ovaries, as well as in HL, NHL, leukemias, neuroblastoma, soft-tissue sarcomas, gliomas and melanoma while the normal tissues from these same organs did not express Survivin[66]. Survivin-directed therapies in animal models and a variety of human clinical trials targeting metastatic melanoma, advanced or recurrent urothelial cancer, metastatic renal cell carcinoma, advanced or recurrent breast cancer, prostate cancer, pancreatic cancer, and non-small cell lung cancer using peptides, peptide-loaded DCs, mRNA vaccines or oncolytic viruses to induce Survivin-specific T cells have revealed no major systemic toxicities[50,67,68,69,70].

(vi) In our own studies targeting NYESO-1, PRAME, and Survivin expressed in lymphoma we have seen no evidence of in vivo toxicities including in individuals with high frequencies of circulating reactive T cells (unpublished results).

Nevertheless, the safety profile associated with targeting these antigens will be established in a dose-escalation manner. We plan on escalating the dose of T cells activated using all four antigens and infused at doses ranging from 5 x 10^6 to 2 x $10^7/m^2$.

Cytokine Release Syndrome:
There have been several reported SAEs associated with cytokine release syndrome (CRS) in patients who received T cells[71] or bispecfic T-cell engagers[72]. The majority of CRS have been reported after the infusion of CAR T cells[73–75], but CRS can also occur after the infusion of

conventional antigen-specific T cells[76] or tumor infiltrating lymphocytes[77]. Patients will be monitored closely as per study calendar and assessed for evidence of incipient CRS (onset of fever, malaise and dyspnea) and treated promptly. Management of CRS will follow published guidelines[71,78], and is described in more detail in SOP F 05.11.XX and includes treatment options based on the clinical severity of the symptoms, such as oxygen, inotropic agents, IL-6 receptor antibody (4-8 mg/kg),TNF-α antibody (5-10 mg/kg), and/or steroids (1-2 mg/kg/day of methylprednisolone or equivalent).

3.0 PATIENT ELIGIBILITY

3.1 Treatment Inclusion Criteria

Patients will be eligible to receive donor-derived multiTAA-specific T cells following any type of allogeneic HSCT as;

(i) Adjuvant therapy for AML/MDS (**Group A)**; or

(ii) Treatment for refractory/relapsed or minimal residual AML/MDS disease **(Group B)**

Residual disease at the time of transplant or post transplant relapse is defined as PCR positivity, specific cytogenetic abnormalities, an abnormal population on flow cytometry or increased blasts on bone marrow biopsy, in the peripheral blood, or any other extramedullary sites.

MRD will be defined as detection in blood, bone marrow, or other tissues any of the following:

a) Any leukemia specific marker such as t(8;21); inv 16; t (15;17), t(9;22) or t(4;11) documented in the patient's leukemia cells pre-transplant on a post-transplant evaluation.
b) Expression of a leukemia associated antigen known to be a marker for residual disease like WT1.
c) A leukemia-specific phenotype (e.g. expression of markers including CD13 and/or CD33 and/or CD117 and/or HLA-DR+) post-transplant at a level of ≥ 0.01%.
d) Mixed donor chimerism (> 20%).

3.1.1 Life expectancy ≥ 6 weeks.

3.1.2 Undergoing Stem Cell Transplant at CAGT

3.1.3 Karnofsky/Lansky score of ≥ 50.

3.1.4 Patient or parent/guardian capable of providing informed consent.

3.1.5 Bilirubin ≤ 2X upper limit of normal.

3.1.6 AST ≤ 3X upper limit of normal.

3.1.7 Serum creatinine ≤ 2X upper limit of normal.

3.1.8 Hgb ≥ 7.0 g/dL (can be transfused).

3.1.9 Pulse oximetry of > 90% on room air.

3.1.10 Sexually active patients must be willing to utilize one of the more effective birth control methods for 6 months after the T cell infusion. Male partner should use a condom.

3.1.11 Available donor-derived multiTAA-specific T cell line.

3.1.12 No other investigational anti-neoplastic therapy for one month prior to entry in this study.

Performance Status Criteria

Karnofsky and Lansky performance scores are intended to be multiples of 10

Karnofsky		Lansky	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed

3.2 Treatment Exclusion Criteria

3.2.1 Patients receiving ATG or Campath within 28 days of infusion.

3.2.2 Patients receiving a Donor Lymphocyte Infusion within 4 weeks of planned T cell infusion.

3.2.3 Less than 30 days post-allogeneic stem cell transplant.

3.2.4 Severe intercurrent infection.

3.2.5 Evidence of GVHD > Grade II.

3.2.6 Pregnant or lactating.

3.2.7 Currently taking corticosteroids (> 0.5 mg/kg/day prednisone or equivalent).

3.3 Informed Consent

The informed consent process will begin at recognition of subject eligibility and consent will be obtained per institutional practices before study therapy is initiated.

3.4 Donor Eligibility

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.X Donor selection, F03.01.X Donor evaluation and F03.05.X Donor Deferral). Subjects must be at least 12 kg or 24 pounds to be eligible for stem cell donation. The stem cell donor will have already been selected by the primary BMT attending according to CAGT SOPs F03.01.X Donor Evaluation, F03.04.X Donor Selection and F03.05.X Donor deferral. If a donor has been chosen for the transplant based on urgent medical need that same donor will also be used for T cell generation provided that there are no new reasons for ineligibility since the stem cell collection. In this study, the subject's stem cell donor will also be the donor for the T cells given in this study. The processes discussed in the protocol and related manufacturing SOPs are in compliance with 21CFR1271. The donors will be evaluated as follows:

3.4.1 Complete history and physical examination.

3.4.2 CBC, platelets, differential.

3.4.3 Electrolytes, BUN, creatinine, glucose, total protein, albumin, total bilirubin, alkaline phosphatase, ALT, AST, LDH, serum protein electrophoresis (if indicated).

3.4.4 HIV-1 antibody, HIV-2 antibody, HIV NAT, HTLV-1/2 antibodies, HBs antigen, HBc antibody, HCV NAT, CMV antibody, RPR, West Nile virus NAT, and Chagas testing.

3.4.5 ABO and Rh typing.

3.4.6 Hemoglobin electrophoresis or Sickle Prep test (if indicated).

3.4.7 Complete urinalysis.

3.4.8 When the evaluation is complete, the transplant physician will note in the recipient's and donors' medical records that the tests have been evaluated, and the donor is acceptable.

3.4.9 Up to an additional 130 mL of blood will be taken from the donor on 1-3 occasions to manufacture the multiTAA-specific T cell line.

3.4.10 In the event that a patient has a donor who is not accessible for a separate procurement, but from whom excess stem cell product was harvested at the time of stem cell collection, the use of the leftover product can be directed by the patient for whom the collection was made and their treating physician. In this situation, on the direction of the treating physician, permission will be sought from the patient for whom the excess product has been cryopreserved to allow it's use as starting material for MultiTAA-T cell manufacture. In this circumstance, since the donor has already undergone the above work-up and as per SOPs F03.04.X Donor selection, F03.01.X Donor evaluation and F03.05.X Donor Deferral has fulfilled the eligibility criteria for stem cell donation at the time of product collection, a second work-up would not be performed.

4.0 STUDY DESIGN

4.1 Design Overview

This is a non-randomized Phase I dose escalation safety and biological efficacy study. We initially elected to use 3 different dose levels starting with 5 x 10^6 cells/m^2, followed by 1 x 10^7 and 2 x 10^7/m^2. However, since we have not reached a maximum tolerated dose level (no DLTs at DL3 to date) we are adding two additional dose levels: 5x10^7 cells/m^2 and 1x10^8 cells/m^2. Each patient will receive a single infusion of cells. If patients with active disease have stable, complete or a partial response by the International Working Group (IWG) criteria (5.5.2, 5.5.3 and 5.5.4) at their 4 week or subsequent evaluations they are eligible to receive up to 6 additional doses of T cells at least 4 weeks apart - each of which will consist of the same cell number as in their initial infusions or below the patient's original dose can be administered. Patients will not be able to receive additional doses until the initial safety profile is completed at 4 weeks following the infusion.

Only patients who can receive infusions in the integrated Cell and Gene Therapy Transplant program at Texas Children's Hospital (TCH) or Houston Methodist Hospital (HMH) are eligible for this study. All cell culture 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 of the manufactured cell product is complete a certificate of analysis will be issued.

4.2 Patient Enrollment

This protocol will be discussed with eligible patients and when appropriate their guardians and informed consent for participation in the study will be obtained. Enrollment of eligible patient/donor pairs will commence prior to stem cell transplant in those patients who are pre-transplant and have an accessible donor. For those patients who have already undergone an alloHSCT, ideally a separate procurement of blood will be obtained from the donor if the donor is accessible (eg: related donors). In the event that a patient has a donor who is not accessible for a separate procurement, but from whom excess stem cell product was harvested at the time of stem cell collection, permission will be sought from the patient for whom the excess product is stored to allow it's use as starting material for MultiTAA-T cell manufacture.

4.3 MultiTAA-Specific T Cell Generation

Generation of multiTAA-specific T-cell lines requires the generation of several different components from peripheral blood mononuclear cells (PBMC) or harvested stem cell product

(SCs). The T-cell line will be derived from donor products (PBMCs, or as part of initial stem cell collection product) by stimulation with APCs pulsed with pepmixes spanning the TAAs WT-1, NY-ESO-1, PRAME and Survivin. The initial stimulation will be performed in the presence of the Th1/pro-proliferative cytokines such as interleukin (IL)-7, IL-12, IL-15, and IL-6, and at second and subsequent stimulations the cells will be expanded in the presence of cytokines such as IL-2 or IL-15. The APCs used to stimulate and expand the multiTAA-specific T cells will be DCs or PBMCs derived from the donor.

For multiTAA-specific T cell generation, we will either use:

- a mononuclear cell-apheresis collection procedure, or
- a maximum blood draw of 130 mL peripheral blood x 1-3 occasions over a two month period to make cells, perform HLA typing if not already performed and to perform infectious disease testing. Subjects must be at least 12 kg or 24 pounds. For pediatric donors the amount will be reduced to 3 mL/kg on each blood draw. PBMCs will be separated from whole blood using ficoll gradients. T cells and monocyte-derived DCs can be prepared from fresh or cryopreserved PBMC.
- Leftover donor peripheral blood stem cell (PBSC) product, that was originally collected for the purposes of transplant but excess PBSCs were cryopreserved.
- Leftover donor marrow stem cell product, that was originally collected for the purposes of transplant but excess product was cryopreserved.

To initiate multiTAA-specific T cell lines, we will make DCs by culture of donor-derived monocytes with cytokines (GM-CSF, IL-4) followed by maturation with a standard DC maturation cocktail (IL-1β, IL-6, TNFα and PGE1). These mature DCs will be pulsed for 30-60 min with a mastermix of pepmixes spanning the target antigens PRAME, WT-1, NY-ESO1 and Survivin. DCs will be pulsed with a mastermix of the pepmixes, then washed once, irradiated and used to stimulate PBMC-derived T cells in the presence of a T cell activating cocktail, IL7, IL-15, IL-12 and IL-6 at a minimum responder:stimulator ratio of 10:1. For initiation, DCs will be prepared from about 100 mL of blood and the T cells will be derived from the non-adherent fraction.

To expand the multiTAA-specific T cells we will use pepmix-pulsed DCs or MCs for the second and subsequent stimulations and cells will be cultured in the presence of IL-2 or IL-15.

At the end of the culture period, T cells will be cryopreserved and aliquots tested for phenotype, function, specificity, identity, sterility and lack of alloreactivity. The frequency of multiTAA-specific T cells will be determined using intracellular cytokine staining, ELIspot assay, and HLA-peptide tetramers, if available. Effector memory phenotype and T cell subsets will be analyzed by flow cytometry.

We will use pepmixes produced by JPT Technologies as an antigen source. These pepmixes are overlapping peptide libraries (15 mers overlapping by 11 amino acids) spanning the entire sequence of each of the antigens of interest. Each peptide has been chemically synthesized to >90% purity (confirmed by HPLC). Once the peptides have been generated they will be compiled into subpools of 10-25 peptides. The presence of all subpool peptides is tracked by LC_MS. A number of peptides within each subpool are defined as "marker peptides". After combining the subpools the final pool is analyzed by LC and MS checking for the "marker peptides" to guarantee presence of all peptides within the final pool.

Products that meet study specific release criteria, as detailed on the CofA, will be infused as per Section 4.5.

If a positive sterility testing result is reported after the product is infused, the FDA and other relevant parties will be notified as per our manufacturing SOP B01.03.XX (Product Quality Assurance Program and Release and Return of Clinical GMP/GTP Products) and clinical research SOP J02.06.XX (Serious Adverse Experience and Unanticipated Problem Reporting). Management of such a situation is further described in our SOP F05.09.XX (Management of Culture Positive Cell Therapy Products).

4.4 Blood Needed for Product Testing

To generate phytohemagglutinin (PHA) blasts to test that donor multiTAA-specific T cells will not attack "normal" recipient tissues, we will take up to 40 mL of blood from the patient. If for any reason it is not possible to obtain the blood from the patient, we will obtain up to 20 mL of blood from a first degree family member after their consent.

4.5 Administration and Monitoring

4.5.1 Timing of Administration

Eligible patients will be evaluated in the clinic and then infused with multi-TAA specific T cells. T cell infusions will be administered from 30 days post-HSCT. A time period of 4 weeks post-infusion will constitute the time for clinical safety monitoring.

4.5.2 Dosing Schedules

This protocol is designed as a phase I dose-escalation study. Three different dosing schedules will be evaluated. Two to four patients will be evaluated on each dosing schedule (Section 6.2).

Each patient will receive one injection at one of these five dose levels: The expected volume of infusion will be 1 to 10 cc.

Dose Level One: 5×10^6 cells/m^2

Dose Level Two: 1×10^7 cells/m^2

Dose Level Three: 2×10^7 cells/m^2

Dose Level Four: 5×10^7 cells/m^2

Dose Level Five: 1×10^8 cells/m^2

4.5.3 Pre-Medication

Patients may be pre-medicated with diphenhydramine (Benadryl) up to 1 mg/kg IV (max 50 mg) and acetaminophen (Tylenol) up to 10 mg/kg po (max 650 mg).

4.5.4 Cell Administration

MultiTAA-specific T cells will be given by intravenous injection over 1-10 minutes through either a peripheral or a central line.

4.5.5 Monitoring of infusion

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.

4.5.6 Supportive Care

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

4.5.7 Sites of Administration

All treatments will be given at the Center for Cell and Gene Therapy in Texas Children's Hospital or Houston Methodist Hospital.

4.5.8 Concurrent Anti-neoplastic Chemotherapy

Ideally, patients should not receive other antineoplastic agents or high dose steroids (>0.5 mg/kg prednisone equivalent) for at least 4 weeks after the T cell infusion. However, patients may receive hypomethylating agents like decitabine and 5-azacytidine, tyrosine kinase inhibitors like sorafenib, mild cytoreductive agents such as hydroxyurea or immunomodulators like lenalidomide which are traditionally not considered to be cytotoxic. If infused patients receive anti-neoplastic treatment or steroids as above the patient will come off treatment and will need to be replaced.

4.5.9 Repeat Dosing

In patients being treated as adjuvant therapy or if patients with residual disease have a complete, partial response or stable disease at the treating physician's discretion they will be eligible to receive up to 6 further doses of multi-TAA specific T cells at least 4 weeks apart at the same dose as the initial infusions or below the patient's original dose can be administered. The calendar (Section 5.3.12) will be reset with each additional dose.

5.0 PATIENT EVALUATION

5.1 History and Physical Examination

A history will be performed at pre-infusion, at 2, 4 and 8 weeks post-infusion, at 3, 6, 9 and 12 months, and then annually for up to 4 additional years for a total of 5 years follow-up. A physical examination will be performed on Day 0. At time points of 3 months and beyond, contact by the investigator or research nurse/research coordinator by phone or email may substitute for history and physical examination if the patient is unable to see a physician or return to clinic.

5.2 Standard Laboratory Studies

5.2.1 The following investigations will be obtained on Day 0 and week 4: CBC and differential, BUN, creatinine, bilirubin, SGOT, SGPT, alkaline phosphatase, Na, K, Cl, CO2, albumin, total protein.

5.2.2 Pregnancy testing is required on female patients of childbearing potential prior to T cell infusion unless they have no possibility of being pregnant (for example post-hysterectomy).

5.3 Immune Reconstitution, MultiTAA-Specific T Cells Persistence and Levels of Disease

5.3.1 Levels of disease will be monitored morphologically by a bone marrow examination and peripheral blood tests which may include flow cytometry, RT-PCR or cytogenetics to identify residual leukemic cells done within 4 weeks prior to infusion, at 4-6 weeks and again at 8-12 weeks post-infusion. The minimum number of time points bone marrow biopsies and peripheral blood samples will be collected will be according to the schedule outlined in the calendar below. However, the treating physician may screen the patient more frequently based on their clinical judgment. Should the patient require other bone marrow biopsies while they are on study a sample of this will also be used to assess their disease status and to perform immune-correlative studies as outlined in 5.3.6.

5.3.2 For patients with a specific cytogenetic abnormality such as t(9;22), inv16, t(8;21), t(15;17), t(4;11), or other complex abnormalities or molecular mutations like FLT3 or NPM1 mutations, disease levels will be assessed using transcript specific Q-PCR or cytogenetic analysis. Detection will be considered consistent with minimal residual disease.

5.3.3 mRNA levels of WT1, PRAME, NY-ESO-1 and Survivin will be assessed prior to infusion and serially post-infusion based on the schedule below (5.3.12).

5.3.4 In those patients with a specific leukemic phenotype, minimal residual disease will be followed using multi-parametric flow cytometry.

5.3.5 Patients with mixed donor chimerism will be followed using FISH and/or STR analysis performed on blood, bone marrow, or other tissues.

5.3.6 The following investigations will be obtained pre-infusion and at weeks 1, 2, 4 and 8 weeks post-infusion and then at 3, 6, 9 and 12 months. Peripheral blood will be collected in preservative-free heparin anticoagulant (20-40 mL) and used for a number of studies including , T cell receptor sequencing for repertoire assessment, and analysis of T cell specificity using HLA-peptide tetramer analysis (when available) and immune function assays including ELIspot, intracellular cytokine staining and cytotoxicity assays. Bone marrow aspirate (5-20cc) will be collected pre-infusion, at 4-6 weeks and again at 8-12 weeks post-infusion and tumor-infiltrating T cells will be subject to the same analyses (T cell repertoire and specificity)depending on availability of cells. Serum will be batched from both blood and bone marrow for analysis of cytokine levels. If applicable, residual blasts will be quantified by morphological or flow cytometric assays and/or WT1 and PRAME mRNA transcript levels will be assessed on peripheral blood/marrow samples by RT-PCR. These studies will be done on patients who have active blasts and if the appropriate reagents are available. In patients with active AML tumor antigen expression, profiling will be performed on isolated tumor cells.

5.3.7 If the patient has additional infusions of cells the follow-up analysis will reset. In this case, the same tests as outlined in Section 5.2 will be performed on blood samples obtained pre each infusion, and at 1, 2, 4 and 8 weeks post infusion. In this case bone marrow sampling will be optional. Follow-up will then continue at 3, 6, 9 and 12 months

after the last infusion. No study-specific blood tests will be done after this time points, but we will continue to follow patients clinically once a year for up to 4 additional years (total of 5 years follow-up) to evaluate long-term disease responses.

5.3.8 If a patient's hemoglobin is less than 7.0 g/dL at any of the evaluation times, the amount of blood/marrow drawn for the evaluation will be reduced.

5.3.9 If there is insufficient blood/marrow for all the tests listed above at any time point the ELISPOT assay will be the first priority.

5.3.10 Studies will be conducted depending on the availability of the patient and the ability to safely draw the amount of blood/marrow needed for the studies. The time points given are approximate as patients may not always be able to keep appointments. However, every effort will be made to obtain studies on the above-mentioned schedule.

5.3.11 Leftover samples will be stored for any future study related assays.

5.3.12 Summary of Monitoring

	Pre-infusion	Day 0	Wk 1	Wk 2	Wk 4	Wk 8	Month 3, 6, 9, 12	Year 2, 3, 4, 5
T-cell infusion		X						
Hx*	X		X	X	X	X	X	X
PE*		X						
Performance Status*		X						
CBC diff		X			X			
Electrolytes and liver function tests**		X			X			
Disease Assessment	X^				X^	X^		
Function and Persistence Studies (includes immune reconstitution studies)#	X^		X	X	X^	X^	X	
Pregnancy Testing#	X							

*At timepoints of 3 months and beyond, contact may be by telephone call or email from PI or research nurse/research coordinator.
**To include: BUN, creatinine, bilirubin, SGOT, SGPT, alkaline phosphatase, Na, K, Cl, CO2, albumin, total protein and LDH.
^ In addition to peripheral blood samples, disease assessment and immune function studies will be performed on blood, bone marrow samples, and/or other tissue samples at these time-points (within 4 weeks pre-infusion, 4-6 weeks and 8-12 weeks post-infusion)
#Study-specific tests
Note – this calendar will reset with each additional dose after the first dose.

5.3.13 Other Tissue: If the patient dies, an autopsy will be requested. If granted, tissue will be requested to assess presence of infused cells and assess tumor antigen expression profile.

Bilateral bone marrow aspirations and other biopsies: Samples of a previous bone marrow biopsy (or other aspirates or biopsies of tissues suspected to be infiltrated with tumor cells) or of additional biopsies done while the patient is on study will be used to assess disease status as well as specificity and functionality of bone marrow infiltrating T cells. For any additional bone marrow biopsy (any done in addition to the protocol specified bone marrow biopsies) the patient has done while they are on study, we will obtain an extra 5-20cc of bone marrow aspirate for the testing described above.

5.4 Toxicity Assessment

5.4.1 The criteria listed in the CTEP NCI Common Toxicity Criteria Scale will be used in grading toxicity (Section 10.3) with the exception of CRS toxicities that are related to T-cell infusions. CRS toxicities will be graded according to Appendix II.

5.4.2 A 4-week period after the infusion will constitute a course, which will be evaluated for critical toxicity, and a period of at least 4-weeks after the T cell infusion will be required for evaluation for antitumor activity. Potential Toxicity for patients receiving allogeneic T cells: Graft versus host disease (GVHD). The risk that adoptively transferred leukemia-targeted T cells will cause GVHD is very low. First, any T cell lines with cytotoxic activity against patient-derived lymphoblasts or skin fibroblasts are excluded from patient use, and secondly, more than 100 patients from our center that have received donor-derived virus-specific T cells post allogeneic HSCT without toxicity.

5.4.3 GVHD Scoring[79]: GVHD will be monitored for 4 weeks following the first T cell infusion. GVHD organ stage scores, overall clinical grade, biopsy information for GVHD and relevant differential diagnosis will be recorded. Organ involvement, biopsy information, staging, differential diagnosis, and GVHD therapy will be documented in the medical record using the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) GVHD scoring stamp or equivalent. A sample assessment is shown below:

Clinical Acute GVHD Assessment

Date _____ Patient ID _____ Karnofsky/Lansky _____

Codes **Differential Diagnosis**

	0	1	2	3	4	5		GVHD	Drug Rxn	Cond Reg	TPN	Infect	VOD	Other
Skin	☐	☐	☐	☐	☐		% body rash:____	☐	☐	☐		☐		_____
Lower GI	☐	☐	☐	☐	☐	☐	Vol: _____	☐	☐	☐	☐	☐		_____
Upper GI	☐	☐						☐	☐	☐	☐	☐		_____
Liver	☐	☐	☐	☐	☐		Max bili: _____	☐	☐	☐	☐	☐	☐	_____

Treatment: ☐ CSA ☐ Tacrolimus ☐ Pred ☐ Methylpred ☐ Ontak
☐ Pentostatin ☐ MMF ☐ Etanercept ☐ Other _____

Code Definitions:

Skin:
0 No rash
1 < 25% of body surface
2 Maculopapular rash, 25-50% of body surface
3 Generalized erythroderma
4 Generalized erythroderma with bullous formation and desquamation

Lower GI (Diarrhea):
0 None
1 ≤ 500 mL/day or < 280 mL/m^2
2 501-1000 mL/day or 280- 555 mL/m^2
3 1001-1500 mL/day or 556- 833 mL/m^2
4 > 1500 mL/day or > 833 mL/m^2
5 Severe abdominal pain with or without ileus, or stool with frank blood or melena

Upper GI:
0 No protracted nausea and vomiting
1 Persistent nausea, vomiting or anorexia

Liver (Bilirubin):
0 < 2.0 mg/dl
1 2.1-3.0 mg/dl
2 3.1-6.0 mg/dl
3 6.1-15.0 mg/dl
4 > 15.1 mg/dl

Signature _____

5.4.4 Chronic GVHD[80]: Chronic GVHD will be assessed at 3, 6 and 12 months post-infusion. Assessment and a description of symptoms (if present) will be documented through completion of the GVHD symptom record (Appendix I).

Organ System	Definite manifestations of chronic GVHD	Possible manifestations of chronic GVHD
Skin	Scleroderma (superficial or fasciitis), lichen planus, vitiligo, scarring alopecia, hyperkeratosis pilaris, contractures from skin immobility, nail bed dysplasia	Eczematoid rash, dry skin, maculopapular rash, hyperpigmentation, hair loss
Mucous membranes	Lichen planus, non-infectious ulcers, corneal erosions/non-infectious conjunctivitis	Xerostomia, keratoconjunctivitis sicca
GI tract	Esophageal strictures, steatorrhea	Anorexia, malabsorption, weight loss, diarrhea, abdominal pain
Liver	None	Elevation of alkaline phosphatase, transaminitis, cholangitis, hyperbilirubinemia
GU	Vaginal stricture, lichen planus	Non-infectious vaginitis, vaginal atrophy
Musculoskeletal/ Serosa	Non-septic arthritis, myositis, myasthenia, polyserositis, contractures from joint immobilization	Arthralgia
Hematologic	None	Thrombocytopenia, eosinophilia, autoimmune cytopenias
Lung	Bronchiolitis obliterans	Bronchiolitis obliterans with organizing pneumonia, interstitial pneumonitis

5.5 Disease Response

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria as indicated in Table 5.5.2, 5.5.3 and 5.5.4. Evaluations of response will be performed at the time points listed in Table 5.3.12. Additional studies (imaging/blood/bone marrow, or other tissue samples know to be involved with leukemia obtained from the patient) performed as part of standard clinical care will also be evaluated. Patient long-term overall and progression-free survival will also be evaluated at 1 year, and then annually for up to 4 additional years for a total long-term follow-up period of 5 years.

5.5.1 This study will use the International Working Group Criteria for MDS and AML. In addition, levels of any molecular marker or mRNA expression of a protein known to represent minimal residual disease or early relapse (eg WT-1), will be serially followed per the schema above. The response criteria are defined below.

5.5.2 Response Criteria in AML[81]

Category	Definition
Complete remission (CR)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 10^9/L (1000/μL); platelet count >100 x 10^9/L (100,000/μL); independence of red cell transfusions
CR with incomplete recovery (CRi)	All CR criteria except for residual neutropenia (<1.0 x 10^9/L (1000/μL)) or thrombocytopenia (<100 x 10^9/L (100,000/μL))
Morphologic leukemia-free state	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	Relevant in the setting of phase I and II clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5 to 25 percent; and decrease of pretreatment bone marrow blast percentage by at least 50 percent
Cytogenetic CR (CRc)	Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow
Molecular CR (CRm)	No standard definition; depends on molecular target
Treatment failure	
Resistant disease (RD)	Failure to achieve CR or CRi (general practice; phase II/III trials), or failure to achieve CR, CRi or PR (phase I trials); only includes patients surviving ≥7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available
Relapse	Bone marrow blasts ≥ 5 percent; or reappearance of blasts in the blood; or development of extramedullary disease

5.5.3 Proposed modified International Working Group response criteria for altering natural history of MDS[82]

Category	Response criteria (responses must last at least 4 wk)
CR	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines*
	Persistent dysplasia will be noted
	Peripheral blood
	Hgb ≥ 11 g/dL

	Platelets ≥ 100 × 10^9/L
	Neutrophils ≥ 1.0 × 10^9/L
	Blasts 0%
PR	All CR criteria if abnormal before treatment except:
	Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%
	Cellularity and morphology not relevant
Marrow CR	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment
	Peripheral blood: if HI responses, they will be noted in addition to marrow CR
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 wks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following:
	Return to pretreatment bone marrow blast percentage
	Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets
	Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete
	Disappearance of the chromosomal abnormality without appearance of new ones
	Partial
	At least 50% reduction of the chromosomal abnormality
Disease progression	For patients with:
	Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts
	5%-10% blasts: ≥ 50% increase to > 10% blasts
	10%-20% blasts: ≥ 50% increase to > 20% blasts
	20%-30% blasts: ≥ 50% increase to > 30% blasts
	Any of the following:
	At least 50% decrement from maximum remission/response in granulocytes or platelets
	Reduction in Hgb by ≥ 2 g/dL
	Transfusion dependence
Survival	Endpoints:
	Overall: death from any cause
	Event free: failure or death from any cause
	PFS: disease progression or death from MDS
	DFS: time to relapse
	Cause-specific death: death related to MDS

5.5.4 Proposed modified International Working Group response criteria for hematologic improvement[82]

Hematologic improvement*	Response criteria (responses must last at least 8 wk)†
	Hgb increase by ≥ 1.5 g/dL

Erythroid response (pretreatment, < 11 g/dL)	Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation
Platelet response (pretreatment, < 100 × 10^9/L)	Absolute increase of ≥ 30 × 10^9/L for patients starting with > 20 × 10^9/L platelets
	Increase from < 20 × 10^9/L to > 20 × 10^9/L and by at least 100%†
Neutrophil response (pretreatment, < 1.0 × 10^9/L)	At least 100% increase and an absolute increase > 0.5 × 10^9/L†
Progression or relapse after HI	At least 1 of the following:
	At least 50% decrement from maximum response levels in granulocytes or platelets
	Reduction in Hgb by ≥ 1.5 g/dL
	Transfusion dependence

6.0 STATISTICAL CONSIDERATIONS

6.1 Study Design

This phase I dose-escalation trial is designed to evaluate the safety and feasibility of a single IV injection of allogeneic multiTAA-specific T cells in patients with AML or MDS. The dose escalation procedures will be operated independently and concurrently within the following 2 patient groups:

Group A: Patients receiving T cells as adjuvant therapy post-transplant for AML or MDS.

Group B: Patients receiving T cells as treatment for refractory/ relapsed or minimal residual disease post-transplant for AML or MDS.

In each group, five dosing schedules with increasing dosages will be studied:

Dose Level One: 5 x 10^6 cells/m^2

Dose Level Two: 1 x 10^7 cells/m^2

Dose Level Three: 2 x 10^7 cells/m^2

Dose Level Four: 5 x 10^7 cells/m^2

Dose Level Five: 1 x 10^8 cells/m^2

The goal is to determine the maximum tolerated dosing schedule (MTD) among the pre-specified dose levels. For this purpose, MTD is defined as the highest dosing schedule at which the probability of DLT is at most 20%.

Except as noted below, dose-limiting toxicity (DLT) is defined as development of grade III-IV GVHD or NCI CTC grade 3-5 toxicity that is ***not*** pre-existing and/or ***not*** due to the underlying malignancy or infection or treatment of disease within 4 weeks of study agent administration.

Grade 3 and 4 expected reactions seen with the use of T cell-based immunotherapy, such as (but not limited to) fever and hypotension not requiring pressor support, will not be considered DLTs. Any other Grade 3 or greater toxicity felt to be related to or resulting from Cytokine Release Syndrome (CRS) is included in the definition of DLT. Grade 3 and 4 CRS infusion reactions (including CRS) that are persistent beyond 72 hours will be reported to the FDA in an expedited fashion, and will be considered a treatment limiting toxicity.

Dose escalation is guided by a modified version of the continual reassessment method (CRM). CRM has been shown in the literature to be superior to the standard 3+3 method designed for the targeted MTD probability 33.3%. To reduce the probability of treating patients at unacceptable toxic dose levels, we employ some modifications to the original CRM (mCRM)[83,84]. Specifically, the modifications are: (1) the first patient starts at the lowest dose level, (2) more than one patient can be treated at the same dose level, (3) there will be no jumps over a dose level and the dose is escalated according to the pre-specified levels, and (4) if a patient experiences a DLT, then there is no dose escalation for the immediate next patient. The modified CRM phase-I design has been studied extensively in the literature. Compared to the 3+3 design, mCRM provides better estimates of the MTD, affords smaller number of patients accrued at lower and more likely ineffective dose levels, and treats more patients at the MTD level[83,84].

Based on our previous experience in similar trials with T cell therapy, we expect the risk of treatment limiting toxicities due to antigen-specific T cell infusion to be very low. Hence, mCRM is implemented using a shallow dose-toxicity curve, represented by logistic model: $prob(Tox| d,\theta)=1/\{1+exp(b - \theta \log(d/c)\}$, where $d=\{0.5, 1, 2\}\times 1e7$, $c=4e7$ and $b=0.41$. Here, θ is the unknown parameter to be estimated based on the data. We assume an informative prior distributed as uniform (0, 2.92) for θ. The prior is chosen such that the prior predictive probability of toxicity is 8.4%, 12.3%, and 21.0%, respectively, of the 3 dose levels.

At the time of Amendment version 6, 18 patients were enrolled to the study and 3 of them were enrolled twice due to disease progression with no other treatment option available. DLT evaluation were only counted once per patient in each group with the worst toxicity outcome. Currently in the ongoing clinical trial, none of the patients have experienced a DLT including the 1 patient enrolled in Group B and the 4 patients enrolled into Group A at Dose level 3 ($2x10^7$ cells/m^2). Based on current observed data with 12 patients in Group A and 6 patients in Group B, the predictive probabilities of toxicity are 1.8%, 4.7%, 14.0% in Group A and 3.5%, 7.0%, 16.4% in Group B, respectively for the first 3 doses. These toxicity probabilities are lower than what we initially expected. So we are now adding two higher doses, 5×10^7 cells/m^2 and 1×10^8 cells/m^2, for testing and expecting their risks are still low. The mCRM design will be used with a logistic model: $prob(Tox| d,\theta)=1/\{1+exp(b - \theta \log(d/c)\}$, where $d=\{0.5, 1, 2, 5, 10\}\times 1e7$, $c=10e7$, $b=0.41$, and θ follows a prior uniform distribution (0, 2.92). The prior predictive probability of toxicity is 4.7%, 5.8%, 7.6%, 12.3% and 21.0%, respectively, of the 5 dose levels.

6.2 Sample Size, Dose Escalation Procedures, and Toxicity Stopping Rules

The study design for enrolling the patients is outlined below. Dose escalation guided by mCRM is conducted in parallel in the two patient groups (Group A and Group B). The dose escalation will be guided by the mCRM method as described above with an accruing cohort of size 2, with a maximum 4 patients in each dose level. When we reach the highest dose level or the MTD level, a total of 6 patients will be accrued at that level to provide additional data on safety. Hence depending on the toxicity outcomes, a minimum of 2 and a maximum of 22 patients will be accrued for each disease group (a range of 4-44 total patients). We will recruit a minimum of 2 patients and up to 44 patients, depending on the toxicity outcomes.

In each patient group, two patients will be initially enrolled to the lowest dose level. During the study, real-time monitoring of patient toxicity outcome will be used to update the posterior toxicity probability. Based on the observed toxicity data, we will update θ by computing its posterior distribution. The predictive probability of DLT for all dose level will be re-evaluated. DLTs that occur during the 4 weeks following the initial injection will be factored into the calculations. Dose escalation in each patient group will be made only after all patients at the current dose levels have completed the necessary 4 weeks DLT evaluation.

The following rule will be applied independently to each patient group. If the predictive probability of DLT of next dose levels is $\leq$ 20%, then a new cohort of 2 patients will be enrolled at the next dose level. If the probability of DLT of next dose level is > 20% but at the current dose level is $\leq$ 20%, then two more patients will be enrolled at the current dose level. If the probability of DLTs at the current dose level is > 20%, the dose will be de-escalated to the highest dose level with predictive probability of DLT $\leq$ 20%. The trial will be stopped if the predictive probability of DLT of all dose levels is > 20% after 4 patients have been studied at current or lower dose levels. The procedure continues until the dose has reached the highest dose level or stopped. If the probability of DLT at the lowest dose level is > 20% after 4 patients, then the trial will be stopped and no MTD will be declared. If the trial reached the highest dose level with the predictive probability of DLT is $\leq$ 20%, then this dose level is tentatively defined as the MTD.

For each patient group, the final MTD will be the highest dose with toxicity probability lower than the target 20%. To ensure safety, we will treat a total 6 patients at the MTD level. As mentioned, the multiTAA-T cell infusion is safe and we do not anticipate seeing any multiTAA-T cell related DLTs. Hence, upon completion of the dose escalation, we anticipate that 10 patients will be treated with total 6 patients accrued at the MTD level for each patient group. If there are one or more DLT events, we expect that at most 22 patients will be enrolled in the phase-I trial for each of two patient groups (total 44).

This study will be complete when the trial is stopped early due to excessive toxicity or a total 6 patients have been studied at the MTD level, whichever comes first. At any time, if there is a treatment-related death, then the trial will be suspended immediately, FDA will be notified, and the clinical data will be further evaluated by the FDA.

6.3 Data Analysis

Safety and toxicity outcomes including DLTs, and laboratory evaluations will be summarized by dose levels using descriptive statistics. Information on the expansion, persistence and anti-tumor effects of the adoptively transferred tumor-specific T cells will be analyzed for the immunological parameters based on multimer analysis, intracellular cytokine staining and ELIspot assays to assess the frequency of cells secreting IFNγ using the descriptive statistics

such as mean, median, standard deviation at each time point. Comparison of MRD measurements will be summarized. Frequencies and proportions of responders will be summarized overall and by dose levels if there are enough patients per dose level.

Growth curves of immune response over time within a patient will be generated to visualize general patterns of immune response. Pairwise comparisons will 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.

All analyses will be performed separately for the two patient groups in this study. In addition, we will combine the data to perform comparisons of immunological responses across dose levels and patient groups in the longitudinal model described above. This combined analysis on more patient numbers will allow us to determine not only safety but also the biologically effective dose to be used for a future Phase II study. The results of this study will not be definitive but only suggestive and a Phase II trial will be undertaken to study the efficacy of the treatment after determining the safe dose level.

For subjects who receive additional infusions, we will collect data on the survival, immunological efficacy and anti-tumor activity of T cell lines after these additional infusions so it may be compared with results obtained after the initial two infusions.

To obtain preliminary data on the safety and feasibility of extended dosing, patients will be monitored for 4 weeks after the final T cell injection for toxicity using standard NIH criteria. If a patient develops Grade III-IV toxicity attributable to the T cell infusions at any time during the extended dosing regimen, they will not be able to receive any more T cell infusions.

7.0 STUDY INTERPRETATION

Since this is a Phase I study, the main aim will be to collect information about the safety, clinical activity, cell dosage required for clinical activity, immunomodulatory effects associated with the infusions (e.g. epitope spreading) and preliminary evidence of antitumor efficacy for a future Phase II study.

It is possible that the tumor-specific T cells will have no significant cytotoxic activity against the tumor. This may because a) the T cells are non-persistent or b) they are persistent but they show no biological activity against the malignant cells. Functional persistence will be measured using tetramer/Elispot/intracellular cytokine staining or cytotoxicity assays.

If patients on the lower dosing schedule fail to show any evidence of disease regression, this would not necessarily indicate lack of efficacy. Rather, this may reflect insufficient numbers of adoptively-transferred T cells or indicate that the dosing frequency is not optimal. Long-term overall and progression-free survival will also be evaluated at 1 year, and then annually for up to an additional 4 years (total of 5 years follow-up).

8.0 MODIFIED FOLLOW-UP AND OFF STUDY CRITERIA

8.1 Criteria for Modified Follow-Up

The following criteria will result in the patient being ineligible for further treatment on the protocol, although response data will continue to be collected as applicable:

8.1.1 Any patient who develops Grade III–IV GVHD or NCI CTC Grade 3 or 4 toxicity primarily related to the T cell infusion, and that is not pre-existing and/or not due to the underlying malignancy or infection or treatment of disease within 4 weeks of study agent administration. In such patients, the toxicities will be followed until resolution or until their off study date.

8.1.2 Any patient who experiences Grade 3 or 4 cytokine release syndrome (CRS) infusion reactions that are persistent beyond 72 hours will be reported to the FDA in an expedited fashion and will be considered a treatment-limiting toxicity. In such patients, the toxicity will be followed until resolution or until their off study date.

8.1.3 Any patient who receives any other hematopoietic cell product except for routine blood product transfusions such as platelets and packed red blood cells. In such patients, adverse event data collection will cease.

8.1.4 Any patient who receives antineoplastic treatment except for treatments listed in Section 4.5.8 for relapse of their primary malignancy within 4 weeks after the T cell infusion. In such patients, adverse event data collection will cease.

Patients who meet modified follow-up criteria will remain on long-term follow-up as per the Summary of Monitoring (see table in Section 5.3.12).

8.2 Off Study

8.2.1 Completion of study-specified procedures.

8.2.2 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.

8.2.3 Lost to follow-up.

8.2.4 Death.

Any questions regarding patients on this study should be addressed to Dr. Lulla at 713-441-1450 or Dr. Heslop at 832-824-4662.

9.0 RECORDS TO BE KEPT

The CAGT research nurse/coordinator will maintain a database documenting on study information, adverse events, off study notification and death information. The dates and doses of

therapy as well as clinical chemistries, 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 Drs. Lulla and Heslop who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of immunological efficacy will be the responsibility of Drs. Leen and Rooney.

10.0 REPORTING REQUIREMENTS

10.1 Register all patients with Cell and Gene Therapy Research Coordinator.

10.2 Enter all patients by phoning Dr. Lulla or Dr. Heslop. The following data will be captured::

Eligibility checklist
Pre-study
Concomitant Medication
Off Study
Adverse Event
CRS Adverse Event (if applicable)
Death

10.3 Drug Toxicity and/or Adverse Reactions

The CTEP NCI Common Terminology Criteria (Version 4.X) for Adverse Events (CTCAE) will be utilized for AE reporting with the exception of CRS toxicities that are related to T-cell infusions. CRS toxicities will be graded according to Appendix II. The CTEP CTCAE (Version 4.X) is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

10.3.1 Toxicity Grading: The criteria listed in the CTEP (Version 4.X) of the NCI Common Toxicity Criteria Scale will be used in grading toxicity in addition to the GVHD scoring scales listed in Section 5.4.3 with the exception of CRS toxicities that are related to T-cell infusions. CRS toxicities will be graded according to Appendix II.

10.3.2 Adverse events will be collected as per SOP J 02.05.XX and J 02.75.XX. Data on adverse experiences/toxicities regardless of seriousness, must be collected for documentation purposes only for 4 weeks after the last dosing of study drug/biologic.

10.3.3 Serious adverse events will be collected as per SOP J 02.06.XX for 1 year after the last infusion.

11.0 INFORMED CONSENT

All patents 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.

12.0 CLINICAL TRIAL OVERSIGHT AND MONITORING

This protocol will be conducted in accordance with the Cell and Gene Therapy Monitoring Plan on file with the FDA and the Dan L Duncan Cancer Center at Baylor College of medicine.

This protocol will be monitored in accordance with the current Data Safety Monitoring Plan of the Dan L Duncan Cancer Center at Baylor College of Medicine.
The conduct of this clinical trial will be evaluated in accordance with the Texas Children's Cancer Center and Center for Cell and Gene Therapy Quality Assurance Policy and Procedure Plan.

13.0 REFERENCES

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APPENDIX I

CHRONIC GVHD ASSESSMENT

Patient's Name:_____

Date of Chronic GVHD Evaluation: _____

Time point of Evaluation (post cell infusion): ☐ Day 90 ☐ Day 180 ☐ Day 365

If the patient does not have symptoms of Chronic GVHD (cGVHD), please mark "None" below and sign and date this page. The assessment table on the attached page does not need to be completed if the patient does not have cGVHD. If the patient does have symptoms of cGVHD, please record the grade and severity below, sign and date this page and complete the assessment table on the attached page.

Maximum Grade of Chronic GvHD: ☐**NONE**

(since the last assessment)

☐**LIMITED** localized skin involvement and/or hepatic dysfunction due to cGVHD

☐**EXTENSIVE** one or more of the following:
--generalized skin involvement; or
--liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or
--involvement of eye: Schirmer's test with < 5 mm wetting; or
--involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or
--involvement of any other target organ

Overall Severity of Chronic GVHD: ☐**NONE**

(since the last assessment)

☐ **MILD** signs /symptoms do not interfere substantially with function and do not progress once appropriately treated with local therapy or standard systemic therapy (corticosteroids and/or cyclosporine or FK506)

☐**MODERATE** signs/symptoms interfere somewhat with function despite appropriate therapy or are progressive through first line systemic therapy (corticosteroids and/or cyclosporine or FK506)

☐**SEVERE** signs/symptoms limit function substantially despite appropriate therapy or are progressive through second line therapy

The following section is to be completed ONLY at the time of the INITIAL ONSET of Chronic GVHD:

Date of Initial Onset of Chronic GVHD: _____
☐Progressive (aGVHD progressed directly to cGVHD)
☐Interrupted (aGVHD resolved, then cGVHD developed)
☐De novo (aGVHD never developed)
☐Chronic GVHD flare (symptoms reactivated within 30 days of drug tapering or discontinuation)

The following section is to completed IF patient has aGVHD symptoms post Day 100:
Did Acute Graft vs. Host Disease (aGVHD) develop or persist (or a flare-up that was more severe) since the date of the last assessment? ☐**YES** ☐**NO**

Additional Information/Comments: _____

_____

_____ _____
Printed Physician Name Physician Signature/Date

Patient's Name:_____

Stage	0	1	2	3
1. Skin	□ No skin changes (a-f)			
a. Rash	□ No changes	□ < 10% of BSA	□ 10 - 50% of BSA	□ > 50% of BSA
b. Lichenoid changes	□ No changes	□ < 10% of BSA	□ 10 - 50% of BSA	□ > 50% of BSA
c. Sclerodermatous changes	□ No changes	□ < 10% of BSA	□ 10 - 50% of BSA	□ > 50% of BSA
d. Hyper or hypopigmentation	□ No changes	□ < 10% of BSA	□ 10 - 50% of BSA	□ > 50% of BSA
e. Dry, flaky skin	□ No changes	□ < 10% of BSA	□ 10 - 50% of BSA	□ > 50% of BSA
f. Alopecia	□ None	□ Mild alopecia	□ Pronounced alopecia	
2. Joints	□ No joint problems (a,b)			
a. Contractures	□ No contractures	--	□ Mild joint contractures (do not affect ADL)	□ Severe joint contractures (affect activities of daily living)
b. Arthralgias/arthritis	□ None	□ Pain and/or inflammation not interfering with function	□ Pain and/or inflammation with either pain or pain meds interfering with function	□ Pain and/or inflammation with either pain or pain meds interfering with activities of daily living
3. Oral	□ No changes	□ Painless erythema and/or lichenoid changes	□ Diffuse erythema, ulcers, and/or lichenoid changes with pain; able to eat most foods	□ Diffuse erythema, ulcers, and/or lichenoid changes with pain; unable to eat most foods
4. Ocular	□ No changes	□ Dryness of eyes, mild, not requiring treatment and/or asymptomatic keratoconjunctivitis	□ Dryness of eyes requiring artificial tears or lacrimal plugging and/or symptomatic keratoconjunctivitis (ie. pain, irritation)	□ Keratoconjunctivitis with pseudomembranes, corneal ulcerations, or loss of vision
5. Hepatic				
a. ALT (SGPT)	□ <= 2.5 x ULN	□ > 2.5 – 5 x ULN	□ > 5 – 20 x ULN	□ > 20 x ULN
b. Bilirubin	□ <= 1.5 x ULN	□ > 1.5 – 3 x ULN	□ > 3 – 10 x ULN	□ > 10 x ULN
c. Alkaline phosphatase	□ <= 2.5 x ULN	□ > 2.5 – 5 x ULN	□ > 5 – 20 x ULN	□ > 20 x ULN
6. Esophagus	□ No dysphagia or odynophagia	□ Mild dysphagia, but can eat regular diet	□ Dysphagia or odynophagia (painful swallowing) requiring dietary changes	□ Dysphagia requiring parenteral nutrition and/or web/stricture formation
7. Gastrointestinal	□ No GI symptoms (a-c)			
a. Vomiting (chronic)	□ No chronic vomiting	□ Vomiting, average 1x/24 hrs x > 1week	□ Vomiting, average 2-5 x/24 hrs x > 1week	□ Vomiting, average ≥ 6x/24 hrs x > 1week
b. Diarrhea (chronic)	□ No chronic diarrhea	□ Increase of 2 -3 stools/day more than normal x > 1week	□ Increase of 4-6 stools/day more than normal, or nocturnal stools x > 1week	□ Increase of ≥ 7 stools/day more than normal, or incontinence, or need for parenteral support for dehydration x > 1week
c. Abdominal pain (chronic)	□ No chronic abdominal pain	□ Mild pain/cramps, not interfering with function	□ Moderate pain/cramps; pain or analgesics interfering with function, but not ADL	□ Severe pain; pain or analgesics interfering with function and ADL
8. Weight loss	□ < 5% weight loss	□ 5-10% loss	□ 10 - <20% loss	□ ≥ 20% loss
9. Pulmonary	□ No pulmonary changes (a-e)			
a. FVC	□ > 75% of predicted	□ ≥ 50 - 75% of predicted	□ ≥ 25 - <50% of predicted	□ < 25% of predicted
b. FEV_1 (+FEV1/FVC<0.8)	□ > 75% of predicted	□ ≥ 50 - 75% of predicted	□ ≥ 25 - <50% of predicted	□ < 25% of predicted
c. $DLCO_2$ (corrected)	□ > 75% of predicted	□ ≥ 50 - 75% of predicted	□ ≥ 25 - <50% of predicted	□ < 25% of predicted
d. Hypoxia	□ Normal	□ ↓ O2 sat with exercise	□ ↓ O2 sat at rest, needs O2	□ Needs CPAP or vent
e. Dyspnea	□ No dyspnea	□ Dyspnea with exertion	□ Dyspnea with normal activities	□ Dyspnea at rest or requiring ventilator support
10. Platelets	□ Platelets ≥ 100,000	--	□ Thrombocytopenia, platelets 50,000-99,000	□ Severe thrombocytopenia, platelets < 50,000
11. Musculoskeletal	□ No serositis or myositis (a,b)			
a. Serositis	□ None	□ Asymptomatic	□ Symptomatic, requiring medical intervention or single aspiration	□ Symptomatic, requiring surgical intervention, drain, or window
b. Myositis	□ None	□ Pain or weakness not interfering with function	□ Pain or weakness interfering with function but not ADL	□ Pain or weakness interfering with activities of daily living
12. Performance (Karnofsky/ Lansky score)	□ 90-100	□ 70-80	□ 50-60	□ <50

Physician Signature: _____ Date: _____

Appendix II - Grading of CRS

CRS Grading Scale

Grade	Symptoms
1	• Symptoms are not life threatening and require symptomatic treatment only (e.g. fever, nausea, fatigue, headache, myalgia, malaise)
2	• Symptoms require and respond to moderate intervention • Oxygen requirement <40% or hypotension responsive to fluids or • low dose of one vasopressor or Grade 2 organ toxicity
3	• Symptoms require and respond to aggressive intervention • Oxygen requirement ≥ 40% or hypotension requiring high dose or multiple vasopressors or • Grade 3 organ toxicity or Grade 4 transaminitis
4	• Life-threatening symptoms • Requirements for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
5	• Death