Coversheet

Protocol and Statistical analysis plan

Official Study Title:	**ADMINISTRATION OF MOST CLOSELY MATCHED THIRD PARTY RAPIDLY GENERATED LMP, BARF1 AND EBMA1 SPECIFIC CYTOTOXIC T-LYMPHOCYTES TO PATIENTS WITH EBV-POSITIVE LYMPHOMA AND OTHER EBV-POSITIVE MALIGNANCIES (MABEL)**
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ADMINISTRATION OF MOST CLOSELY MATCHED THIRD PARTY RAPIDLY GENERATED LMP, BARF1 and EBNA1 SPECIFIC CYTOTOXIC T-LYMPHOCYTES TO PATIENTS WITH EBV-POSITIVE LYMPHOMA AND OTHER EBV- POSITIVE MALIGNANCIES (MABEL)

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1. MABEL CHECKLIST FOR PATIENT ELIGIBILITY AND NECESSARY INFORMATION:

PATIENT ID _____ PATIENT NAME _____

YES	NO	VALUE/DATE	
Any "**NO**" answers will make a patient **ineligible** for study participation.			
			Any patient regardless of age or sex, with diagnosis of either 1) EBV positive Hodgkin's lymphoma 2) EBV positive non-Hodgkin's Lymphoma (regardless of histologic subtype) 3) EBV (associated)-T/NK-lymphoproliferative disease 4) Severe Chronic Active EBV (CAEBV)* 5) Other EBV positive malignancies (e.g. nasopharyngeal carcinoma, smooth muscle tumors, etc.) AND a) in first or subsequent relapse (Group A) or b) with persistent active disease despite therapy (Group B) or c) with active disease if immunosuppressive chemotherapy is contraindicated e.g. patients who develop Hodgkin disease after solid organ transplantation or if the lymphoma is a second malignancy e.g. a Richter's transformation of CLL. (Group C) * CAEBV is defined as patients with high EBV viral load in plasma or PBMC (>4000 genomes per µg PBMC DNA) and/or biopsy tissue positive for EBV
			EBV positive tumor (can be pending at this time)
			Weighs at least 12 kg
			Informed consent obtained from patient/guardian.

Signature of MD _____ Date _____

To check eligibility of a patient, telephone Dr. Rayne Rouce at 832-824-4716. To register a patient, telephone the Center for Cell and Gene Therapy research coordinator at 832-824-4391.

MABEL CHECKLIST FOR PATIENT ELIGIBILITY FOR **TREATMENT**

PATIENT ID _____ PATIENT NAME _____

YES	NO	VALUE/DATE	
Any "**NO**" answers will make a patient **ineligible** for study participation.			
		Please indicate dx 1, 2, 3, 4 or 5 *Please indicate Group a, b, or c.*	Any patient regardless of age or sex, with diagnosis of either 1) EBV positive Hodgkin's lymphoma 2) EBV positive non-Hodgkin's Lymphoma (regardless of histologic subtype) 3) EBV (associated)-T/NK-lymphoproliferative disease 4) Severe Chronic Active EBV (CAEBV)* 5) Other EBV positive malignancies (e.g. nasopharyngeal carcinoma, gastric cancers, smooth muscle tumors, etc.) AND a) in first or subsequent relapse (Group A) or b) with active disease persisting despite therapy (Group B) or c) with active disease if immunosuppressive chemotherapy is contraindicated e.g. patients who develop Hodgkin disease after solid organ transplantation or if the lymphoma is a second malignancy e.g. a Richter's transformation of CLL. (Group C) * CAEBV is defined as patients with high EBV viral load in plasma or PBMC (>4000 genomes per µg PBMC DNA) and/or biopsy tissue positive for EBV
			EBV positive tumor
			Patients with life expectancy ≥ 6 weeks
			Bilirubin ≤ 3x upper limit of normal
			AST ≤ 5x upper limit of normal
			Hgb ≥ 7.0 (may be a transfused value)
			Creatinine ≤ 2x upper limit of normal for age
			Pulse oximetry of > 90% on room air
			Off investigational therapy for at least 30 days at time of infusion.
			Karnofsky or Lansky score of ≥> 50%
			Sexually active patients must be willing to utilize one of the more effective birth control methods during the study and for 6 months after the study is concluded. The male partner should use a condom.
			Informed consent obtained from patient/guardian.

YES	NO	
Any "**YES**" answers will make a patient **ineligible** for study participation.		
		Pregnant or lactating
		Severe intercurrent infection.
		Current use of systemic corticosteroids >0.5mg/kg/day
		Use of ATG, Campath, or other immunosuppressive T cell monoclonal antibodies within 30 days of infusion.

Signature of MD _____ Date _____

To check eligibility of a patient, telephone Dr. Rayne Rouce at 832-824-4716. To register a patient, telephone the Center for Cell and Gene Therapy research coordinator at 832-824-4391.

2. OBJECTIVES

2.1 Primary: To determine the feasibility and safety of administering banked allogeneic, partially HLA-matched rapid EBV Specific T cells (ESTs) in patients with EBV-associated Hodgkin's Disease or non-Hodgkin's lymphoma or T/NK-lymphoproliferative disease and CAEBV.

2.2 Secondary: To assess the anti-viral and anti-tumor effects of ESTs.

2.3 Exploratory:

2.3.1 To determine the survival and the immune function of EST lines.

2.3.2 To obtain preliminary information on the safety and response to an extended dosage regimen.

3. BACKGROUND AND RATIONALE

3.1 Immune Control of EBV Infections

Epstein-Barr virus (EBV) is a gammaherpesvirus that infects more than 95% of the world's population. Primary infection usually produces a mild self-limiting disease, which is followed by latent infection in B cells and productive replication in B cells and mucosal epithelium. There are at least four types of viral latency: type 1, expressing only the virus nuclear antigen 1 (EBNA1); type 2, expressing in addition to EBNA1, the latent membrane proteins, LMP1 and LMP2; and type 3, expressing all seven latency-associated proteins including the immunodominant EBNA3 viral antigens.[1,2] In these types of latency, the viral small RNAs and EBERs are abundantly expressed as well as transcripts from the BamHI A region of the viral genome, BARTs. In individuals with a normal immune response, malignant outgrowth of infected cells is prevented by the mounting of a complex immune response comprised of HLA-restricted EBV-specific cytotoxic T-cells and MHC-unrestricted effectors.[2]

In individuals with a compromised immune system, EBV is potentially oncogenic. EBV-positive lymphoproliferative disease after solid organ or stem cell transplant expresses all of the EBV latent antigens (type 3 latency) and can be fatal.[3] Evidence for control of EBV-positive lymphoproliferative disease post allogeneic bone marrow transplant (BMT) by cytotoxic T lymphocytes (CTL) comes from our study using EBV-specific gene-modified CTL and extension studies at other sites.[4-7] We have recently collated data from the 114 patients entered on these studies in a long-term follow up study.[7] 101 patients received CTLs as prophylaxis (90 after a selectively T cell depleted transplant and 11 with high risk diagnoses). Thirteen patients received CTLs as therapy for EBV lymphoma. None of the patients had any acute Grade 3-5 infusion-related toxicity from CTL infusion. No patients developed de novo GVHD, although 8 of 51 patients with previous GVHD developed a recurrence of disease (grade 1 in 6 patients and grade 2 in 2 patients) all of whom responded to GVHD therapy. The main adverse event was inflammatory response seen in patients with active disease during a therapeutic response in 4 patients, all of whom recovered. None of the 101 patients who received CTL prophylaxis developed EBV- lymphoproliferative disorder (LPD), and 11 of 13 patients treated with CTLs for biopsy-proven or probable LPD achieved sustained complete remissions. The gene-marking component of this study showed the persistence of functional CTLs for up to 9 years. Other studies have confirmed the activity of EBV specific CTLs post-transplant.[8,9]

In the immunocompetent host, other EBV associated tumors have been recognized. However, these express a much more limited range of EBV associated antigens (type I and type II latency). For example, nasopharyngeal carcinomas express EBNA-1, BARF1, LMP1, and LMP2A (latency type II), whereas Burkitt's lymphomas express only EBNA-1 (Latency type I).[10] In EBV-positive Hodgkin's lymphoma and non-Burkitt's non-Hodgkin lymphoma (NHL) the EBV-antigens expressed on the tumor cells are restricted to the expression of the subdominant latent proteins LMP1and LMP2A, BARF1 and EBNA1 (Type II latency). [11]

3.2 Hodgkin's Lymphoma (HL): Background and Association with EBV

HL, a malignant neoplasm of lymphoreticular cell origin, has an incidence of about 2.4 per 100,000 per year[12]. Modern radiotherapy and/or chemotherapy regimens have dramatically improved the cure rate of patients with HL. However, despite the identification of clinical prognostic factors and the optimal use of primary and secondary treatments, HL remains fatal for more than 15% of patients.[13] Even in patients who are cured, the morbidity and excess mortality of therapy are substantial and long lasting.[14-16] New therapeutic agents are therefore required both to further reduce mortality and to alleviate morbidity.

Hodgkin's lymphoma is unusual in that the bulk of the tumor is composed of normal cells within which the malignant Hodgkin-Reed-Sternberg cells, derived from germinal center B cells, are found.[17] In about 40% of HL, tumor cells express viral latency proteins of EBV.[18,19] While more immunogenic proteins (EBNAs and Lytic proteins) are not usually expressed by Hodgkin Reed Sternberg (HRS) cells, EBNA 1, LMP1 and 2 are usually detectable. There are divergent reports in the literature as to whether EBV positivity confers a worse prognosis[19] or has no influence on outcome.[20]

3.3 Non-Hodgkin Lymphoma (NHL): Background and Association with EBV

Non-Hodgkin lymphomas broadly can be divided into B, T, and NK cell categories. Latent EBV infection is associated with a heterogeneous group of NHL, including Burkitt's lymphoma, NK-T lymphomas and B cell diffuse large B cell lymphoma.[21] Most EBV-associated NHLs are aggressive tumors characterized by rapid growth and necrosis, and the NK and T lymphomas in particular are also associated with hemophagocytosis.

3.3.1 B cell NHL

EBV-positive B cell lymphomas that occur in the immunocompetent host include diffuse large B cell lymphoma (DLBCL) and $CD30^+$ anaplastic large cell lymphoma of B cell type. The rate of positivity varies between 5 and 30% in different series depending on criteria for EBV positivity and geographic location.[22] T cell-rich-B cell NHL can be difficult to distinguish from Hodgkin disease, but when strict morphological and immunophenotypic criteria are applied, approximately 20% of these tumors are found to be LMP1 positive.[23]

The 2008 WHO classification also recognizes some DLBCL entities in which the EBV-infected cells may express type 3 latency antigens. These include lymphomatoid granulomatosis, EBV-positive DLBCL of the elderly, and DLBCL associated with chronic inflammation.[24] Large B-cell lymphomas with a phenotype of terminal B-cell differentiation are also often infected by EBV.[25]

3.3.2 T Cell Lymphomas

3.3.2.1 T-NK cell NHL

Extranodal natural killer/T-cell lymphoma, nasal-type, or nasal NK/T-cell lymphoma, was formerly called angio-centric lymphoma. It is an extranodal lymphoma, usually with an immature NK-cell phenotype and positive for Epstein-Barr virus (EBV), with a broad morphologic spectrum, frequent necrosis, and angioinvasion.[26] It is designated NK/T because of uncertainty regarding its cellular lineage. Although originally thought to be T cells, the malignant cells express CD2 and CD56 but lack surface CD3 and T cell receptor gene rearrangements. Thus, these tumors are probably of NK cell origin.

3.3.2.2 Angioimmunoblastic lymphoma

Angioimmunoblastic lymphoma is characterized by oligoclonal proliferation of T and B cells, and EBV is detected in many cases in either T or B cells. The clinical presentation usually includes systemic disease with B symptoms, while pathology reveals a polymorphous infiltrate involving lymph nodes, with a prominent proliferation of endothelial and dendritic cells. Some patients develop a secondary EBV-positive large B-cell lymphoma.

3.3.2.3 Aggressive NK LGL leukemia/lymphoma

This syndrome presents with lymphadenopathy, hepatosplenomegaly, and the presence of atypical lymphocytes in blood and marrow. The phenotype is similar to that of the NK-T lymphomas described above, and the disease is aggressive with a poor prognosis. Infection with EBV has been implicated in more than 50% of the cases of NK LGL leukemia reported in Japan.

3.3.2.4 CAEBV

Another syndrome associated with defective immune control of EBV is severe chronic active EBV (CAEBV) infection syndrome, a rare Epstein-Barr virus associated disorder that may involve T cells, NK cells, or B cells.[27,28] Patients present with fever, hepatosplenomegaly and lymphadenopathy and characteristically have elevated antibody titers to the virus capsid antigen (VCA) and low or absent antibody to EBNA and free virus in serum or other body fluids. This disorder may be complicated by life-threatening complications, such as multi organ failure, chronic interstitial pneumonia and lymphoproliferative disease/lymphoma. The mechanisms responsible for the inability of these patients to produce an effective T cell response to control EBV infection are still unknown and no effective treatment is currently available.

3.4 Nasopharyngeal Carcinoma (NPC): Background and Association with EBV

Nasopharyngeal carcinoma is a malignant disease with a variable range of incidence depending on age, geographical place, race and EBV exposure.[29, 30] The etiological link between NPC and EBV is based on serological evidence, EBV-DNA measurements in plasma[31, 32], and confirmation of EBV-DNA within NPC tumor cells. The EBV-DNA in NPC biopsy samples is clonal, arising from a single EBV infected cell.[33] EBV has been detected in virtually all cases of undifferentiated non-keratinizing NPC.[29, 34, 35]

NPC is a radiosensitive tumor, and by modern radiation techniques, local control rates of greater than 80%are obtained.[36] Despite the high rate of initial remissions with

radiation and concurrent chemotherapy (with 5-year survival rates of 55-70%), distant failures remain the major problem in patients with loco-regionally bulky disease, which is the most common form at presentation due to the lack of early symptoms. Unfortunately, 40-50% of patients still relapse.[37] Even in patients who achieve remission, treatment-related morbidity and mortality are significant.[38, 39] Late medical complications after treatment for NPC include growth hormone deficiency, hypothyroidism and pulmonary fibrosis.[38] Fatal neoplastic complications include secondary leukemia related to alkylating agent chemotherapy and head and neck cancers (most likely radiation-induced), and gastric cancer.[40] It is therefore desirable to develop novel therapies that could improve disease free survival in relapsed/refractory patients and which might ultimately reduce the incidence of long-term treatment related complications in all patients.

The almost uniform expression of the Type II latency EBV antigens in NPC makes it a prime target for immunotherapy with EBV-directed CTLs.[36] We and other groups have previously demonstrated safety and efficacy targeting NPC with EBV-directed CTLs. [41-43]

3.5 Leiomyosarcoma and other Smooth Muscle Tumors: Background Association with EBV

EBV – associated smooth muscle tumors (SMT) and leiomyosarcomas (LMS) are uncommon soft tissue neoplasms typically manifesting in patients with acquired or underlying immune deficiency, including those after solid organ transplant (SOT) and HIV-infected individuals. The association between SMT and immunosuppression was first described in 1970. It was only in 1995 that in 2 simultaneous publications the clear association with EBV was linked. These tumors typically exhibit a Type II latency phenotype.[44]

Therapeutic strategies have historically targeted the tumor location as well as the etiology of immunosuppression. Different treatment modalities have been described for EBV-SMT, including chemotherapy, surgical resection, antiviral therapy, and reduced immunosuppression. However, EBV-associated LMSs are typically aggressive and respond poorly to conventional therapy. Given the rarity and uncertain behavior of these tumors, no fixed approach has been described to treat these tumors.[45]

3.6 Tumor specific CTL as treatment for Lymphoma and other EBV+ Malignancies

3.6.1 Tumor specific CTLs for Lymphoma

LMP1 and 2 are consistently expressed on Reed-Sternberg cells and also detected in NHL tumor cells, which have a type II latency pattern of expression. This therefore represents a potential source of target epitopes. Most donors have a low but measurable frequency of circulating LMP-specific CTL that can be activated and expanded in vitro.[46] Hence LMP1 and 2 may be the proteins of choice to be targeted by CTL in patients with EBV-positive lymphoma, which arises in the immunocompetent host. EBNA1 is also expressed in Type II latency tumors and was initially thought to be a poor target for immunotherapy because it possesses gly-ala repeat sequences that inhibit HLA Class I antigen processing precluding presentation to CD8-positive T cells.[47] More recent studies, however, suggest EBNA1 can still be exploited as a T-cell target.[48, 49] BARF1 is also expressed in some EBV-positive lymphomas as a latent gene.

In our initial immunotherapy studies targeting EBV latency II lymphoma, we generated EBV-specific CTLs in patients with EBV-positive Hodgkin's lymphoma,[50] using lymphoblastoid cell lines (LCL) as EBV-antigen presenting cells (APC). Although these CTLs homed to the tumor sites and produced transient clinical benefit[51], only a small percentage of these CTLs were specific for the antigens expressed by these lymphomas.

In a follow-up study we therefore determined whether anti-tumor activity could be improved by enriching CTL specific for LMP2, an immunologically subdominant antigen that is expressed on these tumor cells.[52] We have manufactured and characterized 25 clinical grade LMP2 lines and infused them into 20 patients in a dose escalation study. After CTL infusion, no immediate or long-term toxicity was observed, and the frequency of EBV + LMP2-specific T cells increased (range 2 to 17.6 fold). This increase persisted for up to 3 months, and represented a 1-3 log expansion of the infused cells in vivo[53]. Of eight patients in remission at the time of CTL infusion, seven remain in remission. Of the eight patients with disease, there were six complete responses, one very good partial response and one progressive disease. Four patients were also treated as adjuvant therapy post allogeneic transplant and only one progressed.

Although these results were encouraging, we hypothesized that broadening the tumor-specificity of CTL would further increase the anti-tumor effect, since tumor antigen loss variants have been observed and the expression of LMP2 in tumors is heterogeneous. We therefore initiated a study in which we manufactured and infused both LMP1 and LMP2-specific CTL to 1) ensure that good CTL epitopes are available regardless of the patient's HLA type and 2) generate a broader CTL response against the malignant cells.

We have recently analyzed the combined results of the studies with LMP2 and LMP1/2 CTLs.

Fifty patients have received autologous LMP CTLs (targeting LMP1/2 (n= 33) or LMP2 (n=17) for NHL or HL), plus an additional 15 patients received donor-derived LMP1/2-CTL (n=11) or LMP2-CTL (n=4) after allogeneic stem cell transplant. In the 15 patients who received donor-derived (allogeneic) CTL, CTL infusions were well tolerated and no de novo GVHD was observed, although one patient developed dermatitis 4 weeks post CTL that has developed into a psoriatic rash of unknown etiology. Twelve of these 15 patients remain in remission for a median of 2 years post CTL (range 3 months-6 years). One patient with HL and 1 patient with NK/T cell lymphoma and 1 patient with T-cell CAEBV progressed within 3 months of T-cell therapy. Of the 50 patients who received autologous LMP-CTL, no toxicity definitely attributable to CTL was seen. Twenty-eight of 29 high-risk or multiple-relapse patients receiving LMP-CTLs as adjuvant therapy remained in remission at a median of 3.1 years after CTL infusion. None subsequently died as a result of lymphoma, but nine succumbed to complications associated with extensive prior chemoradiotherapy, including myocardial infarction and secondary malignancies. Of 21 patients with relapsed or resistant disease at the time of CTL infusion, 13 had clinical responses, including 11 complete responses. T cells specific for LMP as well as nonviral tumor-associated antigens (epitope spreading) could be detected in the peripheral blood within 2 months after CTL infusion, but this evidence for epitope spreading was seen only in patients achieving clinical responses.

3.6.2 Tumor-Specific CTL as Treatment for Other EBV+ Malignancies

Because NPC, gastric cancer, leiomyosarcoma and other EBV+ malignancies are latently infected by EBV, and classified as Latency Types I, II or III, these cancers can also be targeted by EBV-specific CTLs. The expression of Type II latency viral proteins such as EBNA1, LMP1 and LMP2A/B in EBV-associated NPC forms the basis for it's targeting by T cells specifically directed against EBV.[36] Because NPC is highly associated with a latent EBV infection, almost all NPC patients' blood contains a small population of EBV-specific T cells, which can be stimulated ex-vivo to expand into a large population that can be infused back into the patient. We and others have given EBV-specific CTLs to patients with EBV-positive lymphomas and NPC.[41-43]. Autologous EBV-specific CTLs generated from NPC patients' own pool of T cells and directed against EBV antigens such as LMP1/2, as well as EBNA1 and BARF have been investigated by our group at Baylor College of Medicine (NCT00516087, NCT02065362), without acute or long-term dose-limiting toxicities related to T-cell infusions. [41-43] Specifically, we have treated 48 patients with EBV-specific CTLs at our center on three clinical studies. We observed no acute or long-term dose-limiting toxicities related to T-cell infusions.[41-43] Of 30 patients treated with active resistant/relapsed disease, 14 patients (47%) had clinical benefit (5 CR, 2 PR, 7 SD) resulting in a 2-year overall survival (OS) of 43.3%. In contrast, the median 2-year OS for NPC patients with metastatic disease treated on 5 recent clinical studies with chemotherapy was 20%.[54-58] Other groups including pharmaceutical companies have also investigated autologous EBV CTLs as a treatment for NPC even in a large multisite Phase III trial.

To further validate the benefits of EBV-specific CTLs for NPC, we collaborated with investigators at the National Cancer Center in Singapore. NPC is the 6th most common cancer in Singapore and, as everywhere else, the outcome for patients with metastatic disease remains poor. In their study, patients with metastatic NPC received 4 cycles of chemotherapy followed by up to 6 infusions of EBV-specific CTLs. Of 35 patients receiving combined chemotherapy and T-cell therapy, the 2-year OS was 67.2%.[59]

Additionally, there have also been reports of safe and efficacious treatment of EBV-associated SMT/LMS with EBV-directed directed T cells (including those generated from allogeneic donors,[60] (NCT01447056) making this therapeutic approach an option for refractory cases.

Refining the CTL manufacturing strategy

Although the previously mentioned LMP-CTLs have remarkable clinical efficacy, several problems with the current manufacturing strategy limit their broader application. The procedure is long (>12 weeks) and complex. Generation of the requisite EBV-LCL for each patient takes a minimum of 6 weeks, with longer culture required for patients who have received lymphotoxic chemotherapy, while production is impossible in patients who have received the B cell depleting CD20 antibody rituximab, currently the standard of care for patients with B-cell lymphoma. Finally, the use of live EBV to generate LCLs and the requirement for Ad vectors to induce LMP1/2 overexpression both add undesirable cost, and complicate the regulatory process. We have recently been able to remove these viral and viral-

vector components and have shown that T- cells specific for all four type 2 latency proteins can be prepared from healthy donors and lymphoma patients using dendritic cells (DCs) pulsed with peptide libraries (pepmixes) of 15mer amino acids overlapping by 11 that span the entire sequence of the antigens of interest. Responder T-cells can then be expanded by restimulation with the same pepmixes presented on autologous, activated T-cells (AATCs) together with HLA-negative K562 costimulatory cells (K562cs expressing CD80, CD86, CD83 and 4-1BB-ligand as transgenes) (gift from Dr. Carl June). ATCs upregulate HLA class II molecules and therefore can present peptides to CD4+ and CD8+ T-cells, while costimulation is provided in trans by K562cs.

3.6.3 Limitations of stem cell donor-derived tumor specific CTL as treatment for Lymphoma

Despite the successful use of stem cell donor-derived tumor specific CTL as treatment for EBV-positive lymphoma, there are some limitations to this approach. Despite the significant decrease in the amount of time it takes to manufacture EBV-specific T-cell lines with our pepmix-based method (decrease from ~3 months to just 10 days), this time frame is still too long for patients with more fulminant disease. However, the previously recognized limitation from our VST studies that virus specific T lines cannot be generated from seronegative donors should not be an issue considering >90% of the population is EBV seropositive.

3.6.4 Third-party T-cells

Banked third-party “off-the-shelf” EBV-specific T-cells were first used in patients with refractory post-transplant lymphoproliferative disease (PTLD) after solid organ transplantation (SOT)[61]. Tumor responses were seen in 64% of recipients, with a CR in 42%. Doubrovina et al[62] extended this strategy to 25 patients with refractory leiomyosarcoma or PTLD following hematopoietic stem cell transplant (HSCT) or SOT. Sixteen patients (64%) were responders; 9 had CRs and 5 had PRs, while the 2 with leiomyosarcoma had stable disease. Failures occurred when the CTLs were matched with the recipient, but the tumors arose from HLA-mismatched grafts, and in patients with immunodeficiency, implying that reactivation of endogenous recipient T-cells contributes to tumor responses[62].
More recently our group evaluated third-party T-cells specific for EBV, cytomegalovirus and adenovirus in a multicenter trial for patients with therapy-resistant viral infections after HSCT. Lines were generated using adenovector-pp65 transduced monocytes and EBV-LCL and were either retained from our prior donor-specific clinical study or were newly generated from donors with known antiviral activity, including HLA homozygous donors identified by the National Marrow Donor Program. A total of 32 lines were produced and characterized, 18 of which were administered to the 50 study patients. The selection of lines for infusion was based on the specificity of the line for the target virus through a shared HLA allele, as well as the overall level of HLA match.

From the bank of 32 virus specific T-cell lines, a suitable line was identified for 90% of the screened patients within 24 hours. Of 50 recipients, 37 (74%) were partial or complete responders, even when the T-cells were HLA-matched at only a single allele. This included responses in 6 of 9 patients with refractory EBV-PTLD. Only 4 responders had a recurrence or progression of their original disease. Together, these results suggest that third-party T-cells can produce tumor/viral responses that

may be enhanced and sustained by the reactivation of endogenous tumor/virus-specific T-cells. Third-party banks can provide rapid access to virus-specific T-cells for almost any patient, circumventing the delays inherent in activation and expansion of autologous or stem cell donor-derived antigen-specific T-cells.

Additionally, we and others have used third-party EBV CTLs to treat NPC and leiomyosarcoma, demonstrating safety and clinical responses in some patients in an ongoing trial.[60, 62]

3.6.5 The Role of a Lymphodepleted Environment

It is now well established that expansion of lymphocytes *in vivo* is subjected to homeostatic mechanisms that control their total numbers[63]. Therefore, it has become apparent that a certain degree of lymphopenia will facilitate expansion of infused T cells. While lymphopenia will always be present after autologous stem cell transplantation (given the nature of the conditioning regimens, which are myeloablative and lymphodepleting) and in patients who are currently on chemotherapy, subjects who have been off chemotherapy for prolonged periods of time will most likely not be lymphopenic. In this case, induction of lymphopenia before T cell infusion is highly desirable, as evidenced by the much higher rate of complete responses achieved in studies in which high doses of cyclophosphamide and/or fludarabine preceded infusion of chimeric antigen receptor-modified T cells (CAR-T cells).[64-66] We predict that lymphodepletion may be necessary for adequate T cell expansion in patients who have not recently received chemotherapy or undergone stem cell transplant.

3.7 Rationale for Current Protocol

As these previous studies show that T cells directed against the Epstein Barr virus (EBV) latent cycle antigens, LMP1 and LMP2 can produce objective tumor responses in over 50% of recipients, we wish to develop this therapy to licensure. However, as discussed above, the current manufacturing strategy poses several impediments to the required pivotal studies. These include the requirement for live virus (EBV) and viral vectors (adenovirus), the complexity of the procedure and the difficulty or impossibility of generating EBV-transformed B cell line (LCL) for use as antigen-presenting cells (APCs) now that almost all lymphoma patients receive the B cell depleting CD20 antibody, Rituximab, as front-line therapy.

Because of the above problems, we have modified our manufacturing process to eliminate the requirement for EBV-LCLs and adenoviral (Ad) vectors. We have developed a 10-day GMP-compliant T cell production process to generate T cell lines specific for the EBV latent cycle antigens. The first stimulation now uses PBMCs (or dendritic cells (DCs)) pulsed with overlapping peptide libraries representing the entire protein sequences of LMP1, LMP2, BARF 1 and EBNA1 together with the cytokines IL-7 and IL-15. The peptide mixtures ensure that the antigen is effective for any HLA phenotype, and competition between peptides ensures that we select and expand only T cells of sufficiently high receptor avidity to bind to and kill tumor cells. The addition of cytokines to the culture mix prevents activation-induced T cell death by inducing anti-apoptotic molecules, thereby producing a broader antigen-specific T cell repertoire and a higher frequency of antigen-specific T cells. After activation cells are transferred to a G-Rex10 device for 9-11 days and cultured in media containing IL7 and IL15 to achieve maximal

expansion of antigen-specific T cells without carry-over of alloreactive T cells.[67] To prevent compromising the repertoire of the T cells by HLA restriction or lack of CD4+ T cells, the second and subsequent stimulations require additional third-party derived APCs. We use irradiated, third-party derived activated T cells (ATCs) that express both HLA class I and class II molecules, pulsed with the same peptide libraries, together with irradiated HLA-negative K562 cells genetically modified to express a broad range of costimulatory molecules (K562-cs). The responding T cells comprise CD4+ and CD8+ T cells with broad specificity, a high frequency of tumor antigen-specific T cells and a mixed effector phenotype.

In an ongoing clinical trial, 12 patients have been treated with autologous EBNA1/BARF1/LMP specific CTLs manufactured with this method with no toxicity attributable to the infused cells.

The establishment of a third-party “off the shelf” EBV specific CTL bank will directly address the previously recognized drawbacks associated with generating autologous or SCT donor-derived CTLs. Our CTL bank will be generated from eligible HLA typed donors and characterized for specificity, HLA-restriction and function. The banked lines will be chosen for each patient based on their ability to recognize lymphoma antigens through one or more shared HLA alleles. These lines are readily available, and can provide rapid access to EBV-specific T-cells for almost any patient, circumventing the delays inherent in activation and expansion of autologous or stem cell donor-derived antigen-specific T-cell.

3.8 Risks of Administrating LMP-specific CTL

In our studies of LMP CTLs, over 60 patients received autologous LMP CTLs and no immediate or long-term toxicity was observed post CTL infusion.

3.9 Risks of 3rd party CTL

In a study at Memorial Sloan Kettering 35 patients received a median of 6 doses of third party virus specific T cells (VSTs) for EBV after HSCT for EBV LPD, most at 1 x 10^6 EBV-VST/kg/infusion, and all infusions were well tolerated.[62] Updated data from the study was reported at the American Society of Blood and Marrow Transplantation BMT Tandem Meeting 2014, with now 28 patients after HSCT enrolled in this study. Among the 28 patients one patient developed mild skin GVHD after infusion with 3rd party EBV-VSTs, but tolerated a subsequent infusion of EBV-VSTs from an alternate 3rd party donor, with no other incidences of GVHD reported.

In our study of third party VSTs specific for three viruses (AdV, CMV and EBV) a total of 50 patients were infused with third party banked VSTs. All of the infusions were well tolerated. There were no immediate adverse effects, and despite the HLA disparity between the VSTs and recipients, de novo GVHD occurred in only 2 patients (grade I in each case). In the 8 patients in whom acute GVHD developed within 45 days of the first infusion (grade I in 6 patients, grade II in 1 patient, and grade III in 1 patient), 6 had a history of GVHD prior to receiving the VSTs. An additional patient had a flare of chronic skin GVHD. Two patients experienced transplant-associated microangiopathy, a complication that occurs in up to 10% of HSCT recipients, particularly in those receiving sirolimus[68], as were both of our

patients. Only 1 patient had secondary graft failure, concomitant with leukemic relapse.

We have also treated 5 patients with third party LMP1/2 VSTs manufactured using our previous manufacturing methodology (with EBV LCLs and Ad-vectors) with no adverse effects.

3.10 Risk of Cytokine Release Syndrome

There have been several reported SAEs associated with cytokine release syndrome (CRS) in patients who received T cells[69] or bispecific T-cell engagers[70]. The majority of CRS have been reported after the infusion of CAR T cells[71-73], but CRS can also occur after the infusion of conventional antigen-specific T cells[74] or tumor infiltrating lymphocytes[75]. 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[69,76], 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).

3.11 Risks of Lymphodepleting Chemotherapy

3.11.1 Cyclophosphamide

Cyclophosphamide is commercially available. A list of toxicities is attached in Appendix I.

3.11.2 Fludarabine

Fludarabine is commercially available. A list of toxicities is attached in Appendix I.

4. PATIENT ELIGIBILITY

4.1 Eligibility for Screening

4.1.1 Inclusion

4.1.1.1 Any patient regardless of age or sex, with diagnosis of either:

-EBV positive Hodgkin's lymphoma
-EBV positive non-Hodgkin's Lymphoma (regardless of histologic subtype)
-EBV (associated)-T/NK-lymphoproliferative disease
-Severe Chronic Active EBV (CAEBV)*****
- Other EBV positive malignancies (e.g. nasopharyngeal carcinoma, smooth muscle tumors, etc.)

AND
-in first or subsequent relapse (Group A)
-with active disease persisting despite therapy (Group B)
-with active disease if immunosuppressive chemotherapy is contraindicated e.g. patients who develop Hodgkin disease after solid organ transplantation or if the lymphoma is a second malignancy e.g. a Richter's transformation of CLL. (Group C)

***** CAEBV is defined as patients with high EBV viral load in plasma or PBMC (>4000 genomes per µg PBMC DNA) and/or biopsy tissue positive for EBV

4.1.1.2 EBV positive tumor (can be pending)

4.1.1.3 Weighs at least 12kg

4.1.1.4 Informed consent (and assent as applicable) obtained from patient/guardian.

4.2 Eligibility Criteria at time of Treatment

4.2.1 Inclusion:

4.2.1.1 Any patient regardless of age or sex, with diagnosis[#] of either:

-EBV positive Hodgkin's lymphoma
-EBV positive non-Hodgkin's Lymphoma (regardless of histologic subtype)
-EBV (associated)-T/NK-lymphoproliferative disease
-Severe Chronic Active EBV (CAEBV)*****
-Other EBV positive malignancies (e.g. nasopharyngeal carcinoma, smooth muscle tumors, etc.)

AND[#]
-in first or subsequent relapse (Group A)

-with active disease persisting despite therapy (Group B)
-with active disease if immunosuppressive chemotherapy is contraindicated e.g. patients who develop Hodgkin disease after solid organ transplantation or if the lymphoma is a second malignancy e.g. a Richter's transformation of CLL. (Group C)

***** CAEBV is defined as patients with high EBV viral load in plasma or PBMC (>4000 genomes per µg PBMC DNA) and/or biopsy tissue positive for EBV

[#] The disease diagnosis and the Group must be noted on the eligibility checklist.

4.2.1.2 EBV positive tumor

4.2.1.3 Patients with life expectancy ≥ 6 weeks

4.2.1.4 Patients with bilirubin ≤3x upper limit of normal

4.2.1.5 AST ≤ 5x upper limit of normal

4.2.1.6 Hgb ≥ 7.0 (may be a transfused value)

4.2.1.7 Patients with a creatinine ≤ 2x upper limit of normal for age

4.2.1.8 Pulse oximetry of > 90% on room air

4.2.1.9 Patients should have been off other investigational therapy for 30 days prior to infusion.

4.2.1.10 Patients with a Karnofsky/Lansky score of ≥ 50 as described below:

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

4.2.1.11 Sexually active patients must be willing to utilize one of the more effective birth control methods during the study and for 6 months after the study is concluded. The male partner should use a condom.

4.2.1.12 Informed consent (and assent as applicable) signed by patient/guardian.

4.2.2 Exclusion

4.2.2.1 Pregnant or lactating

4.2.2.2 Severe intercurrent infection.

4.2.2.3 Current use of systemic corticosteroids >0.5 mg/kg/day

4.2.2.4 Patients who have received ATG, Campath, or other immunosuppressive T cell monoclonal antibodies within 30 days.

5. STUDY DESIGN

5.1 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. Consent process will consist of two parts: a) screening consent and b) treatment consent

1. Screening consent: The screening portion consists of registering demographic data and patient eligibility data. The search for a suitable matched EST line is initiated if the patient is eligible. If an EST line is not available, the following data will be collected: demographic data, HLA type, and type of diagnosis.
2. Treatment consent: If a suitable matched EST line is available, the principal investigator or designee discusses the available line with the treating physician. After enrollment in the treatment portion of the protocol the patient can then receive the identified EST line if eligibility criteria are still met.

This protocol will be discussed with eligible patients and, when appropriate, with their guardians. Patients who received their primary therapy at another institution will have the protocol discussed when they are referred either to TCH or HMH.

Informed consent for generation of lines and participation in the study will be obtained from eligible third party donors on a separate protocol as denoted below.

All cell culture manipulations will be carried out in the Center for Cell and Gene Therapy CGMP facility using current SOPs. After quality assurance testing is complete a certificate of analysis will be issued.

5.2 Donor Eligibility

The EBV specific T-cell lines that will be used in this third party study are manufactured for third party use from eligible donors with common HLA alleles. Some of these individuals will be subjects who have been enrolled on our IRB approved research study (H -7634) to manufacture CTL lines from normal donors for third party use (including subjects chosen from our panel of highly characterized laboratory donors). We may also collaborate with the National Marrow Donor Program, which is a registry of HLA-typed donors who have volunteered for transplant donation to obtain additional donors. Those donors will be procured on a separate procurement protocol. Alternatively, eligible related donors (parents, siblings) will comprise the remainder of our donor pool.

For all third party lines, donors will be evaluated by the director of the donor center at BCM or an NMDP donor physician for NMDP donors. Donors must meet standard eligibility criteria for donation of blood or marrow. They will be screened with the standard blood bank donor questionnaire, medical history and testing for infectious disease markers by a physician who is experienced in screening transplant donors. Only donors who have cleared this process and are deemed to be eligible will have provided blood for EST generation.

The results of the physician assessment and ID testing will be reviewed by a CAGT laboratory director who gave the final eligibility determination according to the SOPs for Donor Evaluation and Use of Third Party Lines.

5.3 Blood Procurement for CTL and antigen-presenting cell (APC) Generation

5.3.1 CTL Lines selection

Generation of LMP-specific CTL lines requires the generation of several different components from peripheral blood mononuclear cells (PBMC). The CTL line will be derived from third-party donor peripheral blood T cells, by stimulation with antigen-presenting cells (APCs) pulsed with overlapping peptide libraries representing the entire protein sequences of LMP1, LMP2, BARF 1 and EBNA1 and the cytokines IL-7 and IL-15. The APCs used to stimulate and expand the EBV-specific T cells will be derived from donor mononuclear cells and T lymphocytes.

Up to 120 mL of blood will be collected from the donor (subjects must be at least 12 kg). The blood collection may be collected from the donor in 2 draws as needed. For donors <18 years, a maximum of 3mL/kg will be taken over an 8 week period. Peripheral blood mononuclear cells (PBMC) will be harvested using Ficoll gradients. Each component, T cells and dendritic cells, can be prepared from fresh or cryopreserved PBMC. A maximum of 30 mL from adults and 14mL from children will be collected for infectious disease testing (will be collected twice if blood is collected twice for CTL generation) and 9mL will be collected for HLA typing.

To initiate the EBNA1/BARF1/ LMP-specific EST line, we will first manufacture PBMCs (or dendritic cells (DCs)) pulsed with overlapping peptide libraries representing the entire protein sequences of LMP1, LMP2, BARF1 and EBNA1 and the cytokines IL-7 and IL-15. The peptide mixtures ensure that the antigen is effective for any HLA phenotype, and competition between peptides ensures that we select and expand only T cells with receptor avidity sufficient to bind to and kill tumor cells. The addition of cytokines to the culture mix prevents activation-related T cell death by inducing anti-apoptotic molecules. The consequence is a broader antigen-specific T cell repertoire and a higher frequency of antigen-specific T cells.

For second and subsequent stimulations we will use irradiated, donor-derived activated T cells (ATCs) that express both HLA class I and class II molecules, pulsed with the same peptide libraries together with irradiated HLA-negative K562 cells genetically modified to express a broad range of costimulatory molecules (K562-cs). At the end of the EST culture period, the frequency of EBNA1, BARF1 and LMP specific EST will be determined using tetramer reagents if available.

The EST lines will also be checked for identity, phenotype and sterility, and cryopreserved prior to administration, according to SOPs. To test the functional antigen specificity of the EST line we will use individual pepmixes spanning each of the viral antigens used in the initial stimulation as a stimulus in the IFN-Y ELIspot. Epitope mapping will be performed using known HLA epitope peptides or peptide mini-pools to identify novel immunogenic epitope peptides within our target antigens.

Products that meet study specific release criteria, as detailed on the CofA, will be infused as per Section 6.0.

If a positive sterility testing result is reported after the product is infused, the FDA and other relevant parties will be notified as per 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 SOP F05.09.XX (Management of Culture Positive Cell Therapy Products).

5.3.2 No Matched EST Line Available

If no matched line is available the patient will be registered so that the feasibility of the approach can be assessed.

5.3.3 EST Line Available but Patient Status Changes

Patients with a clinical course that changes between screening and infusion and renders patients ineligible at time of infusion will not be given the ESTs.

5.3.4 Criteria for Selection of EST Line

In general, preference will be assigned to the infusion of a line with confirmed virus-specific activity against EBV-specific antigens through a shared HLA allele rather than the overall level of HLA match. For example, a line that matches at 2 loci but that has recognition of EBV mediated through those antigens would be preferable to a line matched at 3 loci but with no demonstrated activity against EBV. Patients with a partial response or stable disease are eligible to receive up to 6 additional doses. Additional doses may be from the same donor or a different donor. Decision to switch to a different donor can be made by the principal investigator based on factors that include non-response to a previous line and/or availability of a better matched or otherwise superior line.

5.4 Administration and Monitoring

Patients will be evaluated in the clinic and 2 doses of EST will be administered, as per **section 6.2**, with or without lymphodepleting chemotherapy (Cy/Flu) as outlined in section 6.1. The decision to administer lymphodepleting chemotherapy (Cy/Flu) will be based on absolute lymphocyte count (ALC) at the time of screening, timing of last chemotherapy and clinical status. Patients with lymphopenia (ALC < 500) due to current drug therapy may be infused at any time starting at least 24 hours after finishing their current cycle of chemotherapy. Patients who have not recently received chemotherapy and are not lymphopenic (ALC≥500) may receive lymphodepleting chemotherapy per **section 6.1**. Patients are not required to reach a target ALC prior to T cell infusion. The decision to administer lymphodepleting chemotherapy will be based on the ALC criterion, but also will take clinical condition and ability to tolerate chemotherapy into account (at the discretion of the PI). Patients will be monitored for clinical toxicity by the NCI Common Toxicity Criteria Scale (Version 4.X located at http://ctep.cancer.gov) with the exception of CRS toxicities that are related to T-cell infusions. CRS toxicities will be graded according to Appendix II. We will also analyze immunological parameters in peripheral blood, including phenotype and EST frequencies by tetramer studies in patients who have HLA types for which such reagents are available. Functional analyses will use cytotoxicity or ELIspot assays. The levels of EBV DNA in peripheral blood before and following infusion will be compared. A time period of 8 weeks after the first dose will constitute the time for

clinical safety monitoring. If patients have had a partial response or have stable disease they will be eligible to receive up to 6 further doses of ESTs, each of which will consist of the same number or less (if there is not enough product available for the subject's original dose) as their second infusion.

6. TREATMENT PLAN

6.1 Dose Levels

The following dose levels will be evaluated. All doses are based on total CD3+ cells/m^2:

Dose Level 1	$2x10^7$ cells/m^2 + $2x10^7$ cells/m^2
Dose Level 2	$2x10^7$ cells/m^2 + $5x10^7$ cells/m^2
Dose Level 3	$5x10^7$ cells/m^2 + $1x10^8$ cells/m^2

In addition, in accordance with the rationale described in Section 5.4:

(a) Patients with lymphopenia (ALC < 500) due to current drug therapy may be infused at any time starting at least 24 hours after finishing their current cycle of chemotherapy.

(b) Patients who have not recently received chemotherapy and are not lymphopenic (ALC $\geq$ 500) may receive 3 daily doses of cyclophosphamide (Cy: 500 mg/m^2/day) together with fludarabine (Flu: 30 mg/m^2) to induce lymphopenia, finishing at least 24 hours before T cell infusion. Patients are not required to reach a target ALC prior to T cell infusion. Infusions should be given following hospital/pharmacy recommendations however at a minimum the cyclophosphamide should be infused over 1 hour and the fludarabine should be infused over 30 minutes. Mesna, IV hydration and anti-emetics should also be provided following local institutional guidelines. As stated in section 5.4, the decision to administer lymphodepleting chemotherapy will be based on the ALC criterion but also will take clinical condition and ability to tolerate chemotherapy into account (at the discretion of the PI).

6.2 Dosing Schedules

Three different dosing schedules will be evaluated. Two to four patients will be evaluated on each dosing schedule.

Each patient will receive 2 infusions, 14 days apart, according to the following dosing schedules. All doses are based on total CD3+ cells/m^2:

Dose Level One:

Day 0	2×10^7 cells/m^2
Day 14	2×10^7 cells/m^2

Dose Level Two:

Day 0	2×10^7 cells/m^2
Day 14	5×10^7 cells/m^2

Dose Level Three:

Day 0	5×10^7 cells/m^2
Day 14	1×10^8 cells/m^2

If patients with active disease have apparent clinical benefit (as determined by symptoms, physical exam, or radiological studies) at their 8 week post 1st infusion (6 weeks after 2nd infusion) or subsequent evaluations they are eligible to receive up to 6 additional doses of ESTs at intervals at least 6 weeks apart each of which will consist of the same cell number as their second injection or below the patient's original dose if there is not enough product available for the subject's original dose. Patients may receive lymphodepleting chemotherapy (Cy/Flu) prior to additional infusions based on guidelines in section 6.1. Patients will not be able to receive additional doses until the initial safety profile is completed at 6 weeks following the second infusion.

6.2.1 Dosing At Lower Dose

Patients may receive dosing at a level lower than the current dose schedule level if an insufficient number of cells have been manufactured and fewer than 4 patients/arm have been treated at that lower level to be consistent with the mCRM statistical design.

6.3 Administration Guidelines

6.3.1 Patients may be premedicated with Benadryl (diphenhydramine) up to 0.25-0.5 mg/kg IV (max 25mg) and Tylenol (acetaminophen) 10mg/kg po (max 650mg).

6.3.2 Cell Administration: ESTs in an expected volume of 1-40cc will be given by intravenous injection over 1-10 minutes through either a peripheral or a central line.

6.3.3 Monitoring will follow institutional standards for administration of blood products with the exception that the injection will be given by a physician.

6.3.4 Patients will receive supportive care for acute or chronic toxicity. Such care will include blood components or antibiotics and other interventions as appropriate.

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

6.3.6 Patients should not receive other treatment for their cancer between the first and second EST doses or for at least 6 weeks post EST (for purposes of evaluation). If a patient receives other treatment for their cancer between the first and second EST doses, that patient will be taken off treatment and will need to be replaced.

6.3.7 If a patient who has had apparent clinical benefit (based on their 8 week post-infusion or subsequent evaluation) receives additional EST doses on the

extended dosing regimen, they should not receive other treatment for their cancer for at least 6 weeks post EST. If a patient receives other treatment for their cancer during this time period, they will be taken off treatment.

7. PATIENT EVALUATION

7.1 Routine Laboratory Investigations

7.1.1 A complete history and physical examination is necessary prior to administration of chemotherapy (if applicable) and ESTs.

7.1.2 The following investigations will be obtained pre-infusion, at 1*, 2, 4 and 6 week(s) post-infusion and then at 3, 6, 9, and 12 months:

- CBC and differential, BUN, creatinine, bilirubin, SGOT, SGPT, Na, K, Cl, CO2, albumin.
- Pregnancy testing is required for female patients of childbearing potential (pre-infusion of chemotherapy or T-cells as applicable).

* At week 1, only a CBC is required.

7.2 Diagnostic Imaging

Diagnostic imaging (CT scans, MRI, nuclear imaging) and/or blood tests (EBV viral load) to document measurable disease and response to therapy will be performed pre-infusion (within 4 weeks prior) and 6 weeks following the second infusion (8 weeks after the first infusion). If diagnostic imaging studies are performed at other times either during or after treatment on this study, that data will be collected and information gained will be used for this study. If the patient receives extended dosing, imaging will be done 1-3 months after the final infusion.

7.3 Immune Function and Persistence Studies

7.3.1 The following investigations will be obtained pre-infusion, at 3-4 days post infusion (optional depending on patient preference), 1, 2, and 6 weeks post-infusion and then at 3, 6, 9, and 12 months.

Peripheral blood is collected in preservative free heparin and ACD (acid citrate dextrose) anticoagulant (20-50ml). This blood will be used for phenotyping of peripheral blood T cells and analysis of specificity of the EST response, using HLA-peptide tetramer analysis and immune function assays including ELISPOT and cytotoxicity assays where appropriate reagents are available. PCR for EBV-DNA will be used to monitor the viral load.

7.3.2 If the patient has additional injections of cells after the first two infusions, the above tests will also be obtained pre each infusion, 3-4 days post each infusion (optional depending on patient preference), and at 1, 2, and 6 weeks post each infusion. Follow up will continue every 3 months until 12 months after the last infusion, then yearly thereafter for 5 years. No study specific blood tests will be

collected after one year (if the patients have had additional injections) but we will remain in contact with patients to follow long term disease responses.

7.4 Other Tissues

7.4.1 Should the patient require a tumor biopsy at any stage during the first year, a sample of this will be used to assess EBV-DNA status.

7.4.2 If a patients' hemoglobin is less than 7.0g/dl, from a previous blood draw, at any of the evaluation times, the amount of blood drawn for the evaluation will be reduced and may be obtained over more than one venipuncture, if necessary.

7.5 Study Calendar

A summary of monitoring studies is provided in the following tables:

7.5.1 For subjects who will not receive lymphodepleting chemotherapy as per guidelines in section 6.1.

Study Procedure	Pre-Study	Treatment							Follow-Up				
		Day 0	Day 3-4	Wk 1	Wk 2	Wk 4	Wk 6	Wk 8	M3	M 6	M 9	M 12	Years 2-5
STUDY DRUG ADMINISTRATION													
EST infusion[a]		X			X								
DATA/TESTS/SPECIMENS TO BE OBTAINED													
Concomitant Medications	X												
Hx		X			X	X	X	X	X	X	X	X	X
PE[b]		X											
Pregnancy Test[c]		X											
Blood Tests [Hematology, clinical chemistry][d]		X		X	X	X	X		X	X	X	X	
Imaging studies[e]	X							X					
Immune function studies[f]		X	X	X	X		X		X	X	X	X	
EBV Viral Load (PCR for EBV)[f]		X	X	X	X	X	X		X	X	X	X	
DATA REVIEW													
Eligibility Assessment for Screening	X												
Eligibility Assessment for Therapy		X											
AE Assessment		X[g]											
DLT Assessment								X					
Response Assessment								X					
Follow-up									X	X	X	X	X

a- Second EST infusion is planned on Day 14, however a delay of up to 2 weeks is allowed if necessary based on clinical status.
b- After initial period of adverse event evaluation patients are followed based on standard of care practice. For the purposes of follow up, after week 8 patients may be followed in clinic or follow up visits may be conducted at the subject's local physicians office with appropriate documentation provided to the research team
c- Pregnancy testing will be done pre-infusion in patients of childbearing potential.
d- Hematology labs for CBC with differential; Clinical chemistry panel includes sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, aspartate transaminase, alanine transaminase, albumin.

e- Evaluations of tumor size will be performed within 4 weeks of beginning treatment and 6 weeks after the second injection to occur anytime between week 6 and week 8.
f- Blood will be used for immune function assays including ELISPOT assays (the day 3-4 post infusion sample is optional)
g- Adverse Events are assessed throughout the clinical safety monitoring timeframe

Additional Treatment

Study Procedure	Treatment					Follow-Up			
	Day 0	Wk 1	Wk 2	Wk 4	Wk 6	M 3	M 6	M 9	M 12
STUDY DRUG ADMINSITRATION									
EST infusion	X								
DATA/TESTS/SPECIMENS TO BE OBTAINED									
Hx	X		X	X	X	X	X	X	X
PE[a]	X								
Pregnancy Test[b]	X								
Blood Tests [Hematology, clinical chemistry and lipase][c]	X	X	X	X	X	X	X	X	X
Imaging studies[d]									
Immune function studies[e]	X	X	X		X	X	X	X	X
EBV Viral Load (PCR for EBV) [e]	X	X	X	X	X	X	X	X	X
DATA REVIEW									
AE Assessment	X[f]								
Response Assessment					X[d]				
Follow-up						X	X	X	X

a- After initial period of adverse event evaluation patients are followed based on standard of care practice. For the purposes of follow up, after week 8 patients may be followed in clinic or follow up visits may be conducted at the subject's local physicians office with appropriate documentation provided to the research team
b- Pregnancy testing will be done pre-infusion in patients of childbearing potential. Either serum or urine is acceptable
c- Hematology labs for CBC with differential WBC count; Clinical chemistry panel includes sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, aspartate transaminase, alanine transaminase, total protein, and Lipase.
d- Imaging will be done 1-3 months after the final EB-VST infusion.
e- Blood will be used for immune function assays including ELISPOT assays
f- Adverse Events are assessed until 6 weeks after the last dose of study drug

7.5.2 For subjects who will receive lymphodepleting cyclophosphamide and fludarabine (as per guidelines in section 6.1).

Study Procedure	Pre-Study	Treatment								Follow-Up				
		Day -4	Day 0	Day 3-4	Wk 1	Wk 2	Wk 4	Wk 6	Wk 8	M3	M 6	M 9	M 12	Years 2-5
STUDY DRUG ADMINISTRATION														
Cytoxan/Fludarabine		X												
EST infusion[a]			X			X								
DATA/TESTS/SPECIMENS TO BE OBTAINED														
Concomitant Medications	X													
Hx		X	X			X	X	X	X	X	X	X	X	X
PE[b]		X	X											
Pregnancy Test[c]		X												
Blood Tests [Hematology, clinical chemistry][d]		X	X		X	X	X	X		X	X	X	X	
Imaging studies [e]	X								X					
Immune function studies[f]		X	X	X	X	X		X		X	X	X	X	
EBV Viral Load (PCR for EBV) [g]			X	X	X	X	X	X		X	X	X	X	
DATA REVIEW														
Eligibility Assessment for Screening	X													
Eligibility Assessment for Therapy			X											
AE Assessment		X[f]												
DLT Assessment									X					
Response Assessment									X					
Follow-up										X	X	X	X	X

a. **Second EST infusion is planned on Day 14, however a delay of up to 2 weeks is allowed if necessary based on clinical status.**

b. **After initial period of adverse event evaluation patients are followed based on standard of care practice. For the purposes of follow up, after week 8 patients may be followed in clinic or follow up visits may be conducted at the subject's local physicians office with appropriate documentation provided to the research team**

c. **Pregnancy testing will be done pre-infusion in patients of childbearing potential.**

d. **Hematology labs for CBC with differential; Clinical chemistry panel includes sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, aspartate transaminase, alanine transaminase, albumin.**

e. **Evaluations of tumor size will be performed within 4 weeks of beginning treatment and 6 weeks after the second injection to occur anytime between week 6 and week 8.**

f. **Blood will be used for immune function assays including ELISPOT assays (the day 3-4 post infusion sample is optional)**

g. **Adverse Events are assessed throughout the clinical safety monitoring timeframe**

Additional Treatment

Study Procedure	Treatment						Follow-Up			
	Day -4	Day 0	Wk 1	Wk 2	Wk 4	Wk 6	M 3	M 6	M 9	M 12
STUDY DRUG ADMINSITRATION										
Cytoxan/Fludarabine	X									
EST infusion		X								
DATA/TESTS/SPECIMENS TO BE OBTAINED										
Hx	X	X		X	X	X	X	X	X	X
PE[a]	X	X								
Pregnancy Test[b]	X									
Blood Tests [Hematology, clinical chemistry and lipase][c]	X	X	X	X	X	X	X	X	X	X
Imaging studies[d]										
Immune function studies[e]		X	X	X		X	X	X	X	X
EBV Viral Load (PCR for EBV) [e]		X	X	X	X	X	X	X	X	X
DATA REVIEW										
AE Assessment	X[f]									
Response Assessment						X[d]				
Follow-up							X	X	X	X

a- After initial period of adverse event evaluation patients are followed based on standard of care practice. For the purposes of follow up, after week 8 patients may be followed in clinic or follow up visits may be conducted at the subject's local physicians office with appropriate documentation provided to the research team

b- Pregnancy testing will be done pre-infusion in patients of childbearing potential. Either serum or urine is acceptable

c- Hematology labs for CBC with differential WBC count; Clinical chemistry panel includes sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, aspartate transaminase, alanine transaminase, total protein, and Lipase.

d- Imaging will be done 1-3 months after the final EB-VST infusion.

e- Blood will be used for immune function assays including ELISPOT assays

f- Adverse Events are assessed until 6 weeks after the last dose of study drug

8. EVALUATION DURING STUDY

8.1 Follow up Interval

Patients shall be evaluated (seen in clinic or contacted by research coordinator) at two-week intervals for the first eight weeks, then at 3, 6, 9 and 12 months, then yearly for 5 years. Additional visits will be obtained as clinically indicated or if the patient is having more than 2 infusions.

8.2 Early Termination of Study and Modifications of Drug Dosages

8.2.1 The study will be completed when all dose levels have been safely studied (see Section 9) in each group, OR the study has reached the maximum tolerated dose lower than dose level 3.

8.2.2 The criteria listed in the NCI Common Toxicity Criteria Scale will be used in grading toxicity. (Version 4.X located at http://ctep.cancer.gov) with the exception of CRS toxicities that are related to T-cell infusions. CRS toxicities will be graded according to Appendix II.

8.2.3 A 6-week period after the second infusion (8 weeks after the first infusion) will constitute a course, which will be evaluated for critical toxicity and evaluation for antitumor activity. If a patient receives other treatment for their cancer between the first and second EST doses, we will discontinue study agent administration. In that case, our study follow up will be limited to long term outcome.

8.3 Measurement of Effect

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. Evaluations of tumor size will be performed within 4 weeks of beginning treatment and 6 weeks after the second injection (8 weeks after the 1st infusion). All patients who receive the first infusion will be evaluable for response (aside from those who receive chemotherapy between the first and second infusion).

8.3.1 Measurement of Disease

This study will use the International Working Group Criteria **(*Cheson BD, Annals Oncol 2008;19(Suppl 4):iv35)*** for measurement of disease in patients with lymphoma***.***

The response criteria are defined below

Response	***Definition***	***Nodal masses***	***Spleen/liver***	***Bone Marrow***
Complete remission (CR)	Disappearance of all evidence of disease	(a) FDG avid or PET+ before therapy: mass of any size permitted if PET–;	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy, if indeterminate by morphology immunohistochemistry should be negative

		(b) variably FDG avid or PET–: regression to normal size on CT		
Partial remission (PR)	Regression of measurable disease and no new sites	‡50% decrease in SPD of up to six largest dominant masses. No increase in size of other nodes (a) FDG avid or PET+ before therapy: one or more PET+ at previously involved site; (b) variably FDG avid or PET–: regression on CT	‡50% decrease in SPD of nodules (for single nodule in greatest transverse diameter), no increase in size of liver or spleen	Irrelevant if positive before therapy, cell type should be specified
Stable disease (SD)	Failure to attain CR/PR or PD	(a) FDG avid or PET+ prior to therapy: PET+ at prior sites of disease and no new sites on CT or PET; (b) variably FDG avid or PET–: no change in size of previous lesions on CT		
Relapsed or progressive disease	Any new lesion or increase from nadir by ‡50% of previously involved sites	Appearance of a new lesion>1.5 cm in any axis ‡50% increase in the longest diameter of a previously identified node >1 cm in short axis or in the SPD of more than one node; lesions PET+ if FDG-avid lymphoma or PET+ before therapy	‡50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

In patients with CAEBV, response and progression will be evaluated by decreases in EBV viral load in blood and other tissues as applicable.

This study will use the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee[77] for measurement of disease in patients with solid tumors (NPC, leiomyosarcoma, SMT).

Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques (CT, MRI, x-ray) or as >10 mm with spiral CT scan. The investigator will identify up to 10 measurable lesions to be followed for response. Serial measurements are to be done with CT or MRI. The same method of assessment is to be used to characterize each identified and reported lesion at baseline and during follow-up.

Complete Response: Disappearance of all target lesions

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design Synopsis

This protocol is designed as a phase I study to evaluate the safety and clinical response of 2 IV injections of rapid EBV Specific T cells (ESTs) in patients with EBV-associated Hodgkin's Disease, lymphoma, CAEBV, or other EBV-associated malignancies. This is a phase-I dose-escalation trial to determine the maximum tolerated dose (MTD) level. The three dose levels of interest are discussed in **Section 6.2**. The dose escalation procedures will be operated separately and concurrently for the following 3 patient groups

Group A:
Patients in first or subsequent relapse

Group B:
Patients with persistent active disease despite therapy

Group C:
Patients with active disease if immunosuppressive chemotherapy is contraindicated e.g. patients who develop Hodgkin disease after solid organ transplantation or if the lymphoma is a second malignancy e.g. a Richter's transformation of CLL.

We expect the safety profile to be similar across the different disease types, including NPC, leiomyosarcoma, and other smooth muscle tumors, as EBV-specific T cells have been used in the past in these disease groups. We therefore do not feel the addition of patients with other EBV+ malignancies in the middle of the dose escalation will change the safety profile. As of the date of this amendment, all groups have finished dose level 1 without any DLT. The addition of patients with non-lymphoma EBV-associated malignancies will start with dose level 2 in group B.

9.2 Sample Size and Design Characteristics

Dose escalation in each patient group will be guided by a modified continual reassessment method (mCRM) to determine the MTD described as follows. The toxicity will be evaluated as per the NCI CTC Version 4.X and **Section 8.2** of the protocol. Dose limiting toxicity (DLT) is defined in **Section 9.3**. MTD is defined as the highest dose level at which the probability of DLT is at most 20%.

Based on our previous trials, we expect the risk of toxicity due to EST infusion to be very low. To find the MTD with the targeted probability of 20%, we will implement the CRM method to guide the dose escalation, which 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 also employ some modifications to the original CRM. 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.[78-80]

During the dose escalation, each patient will be followed for 6 weeks post the 2nd EST infusion for the evaluation of DLTs. Two patients can be treated concurrently at the same dose level while the maximum number of patients in each dose level is 4. As we expect the risk of DLT due to EST infusion to be very low, we feel comfortable with the slightly accelerated dose-escalations (2 subjects in each cohort) without compromising patient safety. Depending on DLT data at each dose level, maximum 14 patients will be accrued for each patient group, where we will enroll 6 patients at the MTD dose level to ensure its safety.

The dose escalation guided by a model-based mCRM will be based on a logistic model for the probability of toxicity $Prob(Tox|d)=1/\{1+exp(0.41- \theta \log[(d_a+d_b)/\alpha]\}$, where d_a+d_b is the sum of 2 IV injected cells ($0.4x10^8$, $0.7x10^8$, $1.5x10^8$, respectively), $\alpha=3x10^8$ is a scaling constant, and θ is the unknown parameter. We will implement a Bayesian version of the CRM and assume a non-informative uniform prior for θ. Based on our previous trials, we expect a shallow dose-toxicity curve. The prior predictive probability is chosen conservatively to be 8.6%, 11.8%, and 21% for the three dosing schedules, respectively.

Two patients will be initially enrolled to the lowest dose level. Based on the observed toxicity data from the two subjects, we will update θ by computing its posterior distribution. The predictive probability of DLT under the current dose level and the next dose level are then evaluated. DLTs that occur during the 8 weeks following the initial injection will be factored into the CRM calculations to determine the predictive probability, so the next cohort of patients in each patient group will wait until the completion of the 8 weeks DLT evaluation. During the study, real-time monitoring of patient toxicity outcome will be performed in order to estimate the dose-toxicity curve.

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 under 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% and 4 patients have been studied, then this dose level is tentatively defined as the MTD. Two more patients will be enrolled at this dose level (total 6 at the MTD dose level).

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 EST is safe and we do not anticipate seeing any EST-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 indeed one or more DLT events, we expect that at most 14 patients will be enrolled in the phase-I trial for each of three patient groups (total 42). If there is any death possibly or probably related to EST infusion, then the study will be halted for patient accrual for the respective patient group, and the data will be discussed with the FDA.

9.3 Dose Limiting Toxicity

Except as noted below, a dose limiting toxicity is defined as any toxicity that is considered to be primarily related to the EST infusion that is irreversible, life threatening or a non-hematologic Grade 3-5.

Grade 3 and 4 expected reactions seen with the use of T cell-based immunotherapy, such as fever and hypotension 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 that are persistent beyond 72 hours will be reported to the FDA in an expedited fashion, and will be considered a treatment-limiting toxicity.

9.4 Data Analysis

9.4.1 Safety Analysis of Adverse Event Data

All patients who received EST infusions will be included in the safety analysis. All analyses will be performed separately for the three patient groups in this study. Safety and toxicity outcomes will be summarized for the overall patient group and by dose levels. Adverse event data and corresponding toxicity grades six weeks after EST infusions and during long-term follow-up will be summarized in the form of tables. Incidence tables will be generated to summarize incidence of patients reporting at least one episode of each specific adverse event, incidence of adverse events causing withdrawal and incidence of serious adverse events. The total number of episodes for each event reported (Frequency Table), and the severity and attribution to study therapy of each episode reported (Severity Table and Attribution Table) will also be displayed.

Listings of adverse events by patients will include the time to onset, the duration of each event, the severity of each event, and the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal. Safety data will be summarized.

9.4.2 Clinical response data

For each patient group, response rates will be estimated as the percent of patients whose best response is either complete remission or partial remission (**see Section 8.3**) by combining the data from the three patient groups. To compare with historical data, a 95% confidence interval will be calculated for the response rate.

The analysis of time to progression will include all patients who are considered evaluable for response. The probability of progression free survival at 6 months will be summarized. Time to progression will be estimated using the product-limit method of Kaplan and Meier, and their associations with biologic biomarkers will be studied by the Cox regression in an exploratory way. Time to progression is defined as the number of days from enrollment to: (1) disease progression; (2) death because of treatment complications; or (3) last patient follow-up whichever is first. Patients will be considered to have experienced a progression event if (1) or (2) occurs. Otherwise, the patient will be considered censored for time to progression.

9.4.3 Safety Analysis of Laboratory Data

Descriptive statistics (means, standard deviations, medians and ranges) at pre-infusion and at 1, 2, and 6 and 8 weeks post-EST infusion will be calculated. Laboratory data collected at 3-month intervals for the first year will also be summarized. Scatter plots depicting laboratory values at each time point for each patient will also be generated. In order to analyze changes in laboratory values, a shift table with Stuart-Maxwell chi-square analysis of the change in the normal range from pre-infusion to post infusion time points (using high, normal, low) will be performed. When appropriate, these tables are collapsed and the McNemar's test applied in place of the Stuart-Maxwell test.

9.4.4 Analysis of Expansion and Persistence of ESTs

Despite the small patient numbers, a data-dense study will be generated due to the repeat measurements on proliferation, immune function, etc. on each patient. Descriptive statistics using mean ± SD, medians and ranges will be calculated to summarize repeated measurements of EST expansion (increasing EST levels in the peripheral blood over time), persistence (infused ESTs continuing at detectable levels over time), and function (ability to respond to viral antigens) following infusion. Immune response to viral antigens measured by interferon-γ (IFNγ) ELISPOT will be analyzed. Plots of growth curves will be generated to graphically illustrate patterns of survival and expansion of ESTs as well as immune response. The validity of the normality assumption on these data will be tested and appropriate transformations will be considered whenever indicated.

To compare the persistence of ESTs among the three dose levels, we will calculate the area under the growth curves (AUC) over time for EST frequencies. After appropriate log-transformation, ANOVA and t-test will be used to compare the AUCs with Tukey adjustment for multiple comparisons. Alternative strategies such as repeated measurement models will be applied to compare immunological and virological efficacy across dose levels. We will use a type-I error of 10% in the analysis because of the small sample size.

The modeling strategies proposed here are amenable to these types of data but will however be considered exploratory and interpreted with caution due to limited study power and high type-I error. The results of this study will not be definitive but only exploratory.

10. MODIFIED FOLLOW - UP and OFF-STUDY CRITERIA

10.1 Modified Follow-Up Criteria:

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:

10.1.1 Any patient who develops irreversible, life threatening or non-hematologic Grade 3 to 5 toxicity considered to be primarily related to EST infusion. In such patients the toxicities will be followed until resolution or until their off study date.

10.1.2 Any patient who receives any other hematopoietic cell product. In such patients adverse event data collection will cease.

10.1.3 Any patient who receives therapy for relapse of their primary malignancy. In such patients adverse event data collection will cease,

10.1.4 Any patient who experiences Grade 3 or 4 cytokine release syndrome that persists beyond 72 hours. In such patients, the toxicity will be followed until resolution or until their off study date.

10.1.5 Any patient who receives other treatment for their cancer between the first and second EST doses will be taken off treatment and will need to be replaced.

Patients who meet modified follow up criteria remain on long term follow-up as per study calendar (Section 7.5).

10.2 Off Study Criteria

Patients are considered to be off study if they meet the following criteria:

10.2.1 Completion of study specified procedures
10.2.2 Refusal of further study follow up by patient or legal guardian
10.2.3 Lost to follow up
10.2.4 Death

Any questions regarding patients on this study should be addressed to Dr. Rouce at 832-824-4716.

11. 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 Dr. Rouce, who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of immunological efficacy and EBV DNA persistence will be the responsibility of Dr. Rooney.

12. REPORTING REQUIREMENTS

12.1 Registration

- Register all patients with Cell and Gene Therapy Research Coordinator.
- Enter all patients by phoning Dr. Rouce. The following data should be collected:

  Eligibility
  On study
  Concomitant medications
  Off study
  Flow sheets
  Adverse events
  CRS adverse event (as applicable)
  Death (if applicable)

12.2 Drug Toxicity and/or Adverse Reactions

12.2.1 Adverse events will be collected as per SOP J 02.05.XX and SOP J 02.75.XX. Data on adverse experiences/toxicities regardless of seriousness, must be

collected for documentation purposes only for 6 weeks after the last dosing of study drug/biologic.

12.2.2 Serious adverse events will be collected for 1 year post-last infusion as per SOP J 02.06.XX.

13. INFORMED CONSENT

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

14. CLINICAL TRIAL OVERSIGHT AND MONITORING

This protocol will be conducted in accordance with the Cell Therapy Monitoring Plan on file with the FDA.

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.

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Appendix I

Potential Side Effects of Lymphodepleting Chemotherapy

Risks and side effects related to <u>CYCLOPHOSPHAMIDE</u> include those which are:

Likely	Less likely	Rare but serious
• Loss of appetite • Nausea • Vomiting • Fewer white blood cells • in the blood. • A low number of white blood cells, which may make it easier to get infections. • Hair loss • Decreased ability of the body to fight infections • Absence or decrease in the number of sperm, which may be temporary or permanent which may decrease the ability to have children	• Abnormal hormone function which may lower the level of salt in the blood • Abdominal pain • Diarrhea • Fewer red blood cells and platelets in the blood • A low number of red blood cells may make you feel tired and weak. • A low number of platelets may cause you to bruise and bleed more easily. • Bleeding and inflammation of the urinary bladder • Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children • Temporary blurred vision • Nasal stuffiness with IV infusions • Skin rash • Darkening of areas of the skin and finger nails • Slow healing of wounds • Infections	• Heart muscle damage which may occur with very high doses and which may be fatal. • Abnormal heart rhythms • Damage and scarring of lung tissue which may make you short of breath • A new cancer or leukemia resulting from this treatment. • Damage or scarring of urinary bladder tissue • Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever • Infertility, which is the inability to have children

Risks and side effects related to <u>FLUDARABINE (IV)</u> include those which are:

Likely (May happen in more than 20% of patients)	**Less Likely** (May happen in fewer than 20% of patients)	**Rare, but Serious** (May happen in fewer than 2% of patients)
• Low number of red blood cells (anemia) • Low number of white blood cells • Low number of blood platelets • Feeling tired • Nausea (feeling sick to your stomach) • Throwing up (vomiting) • Weak immune system • Pneumonia • Infection • Bleeding • Pain • Electrolyte imbalance	• Diarrhea • Mouth sores • Skin rash • Fever • Swelling of hands and feet • Numbness and tingling in hands and/or feet • Loss of appetite	• Changes in vision • Feeling nervous or anxious • Confusion • Cough • Difficulty breathing • Feeling weak • Severe brain injury which can lead to death • Kidney damage that could require dialysis • Coma • New (secondary) cancers

Appendix II 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