A Phase II Study of the Therapeutic Effects of Epstein-Barr Virus Immune T-Lymphocytes Derived from a Normal HLA- Compatible or Partially- Matched Third-Party Donor in the Treatment of EBV Lymphoproliferative Disorders and EBV-Associated Malignancies

PROTOCOL FACE PAGE FOR MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	PPD, M.D.	Pediatrics, BMT
Co-Principal Investigator(s)/Department:	PPD, M.D.	Medicine, BMT
Investigator(s)/Department:	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT
	PPD, M.B.B.S.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D., Ph.D,	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, MD	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, Head & Neck
	PPD, M.D.	Medicine, Head & Neck
	PPD, M.D.	Medicine, Head & Neck
Consenting Professional(s)/Department:	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT

	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT
	PPD, M.D.	Pediatrics, BMT
	PPD, M.B.B.S.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, BMT
	PPD, M.D.	Medicine, BMT
	PPD, M.D., Ph.D.	Medicine, Head & Neck
	PPD, M.D.	Medicine, Head & Neck
	PPD, M.D.	Medicine, Head & Neck
	For Donor Consents and Patient Assessment Consents ONLY:	
	PPD	Medicine, BMT
	PPD	Pediatrics, BMT
	PPD	Pediatrics, BMT

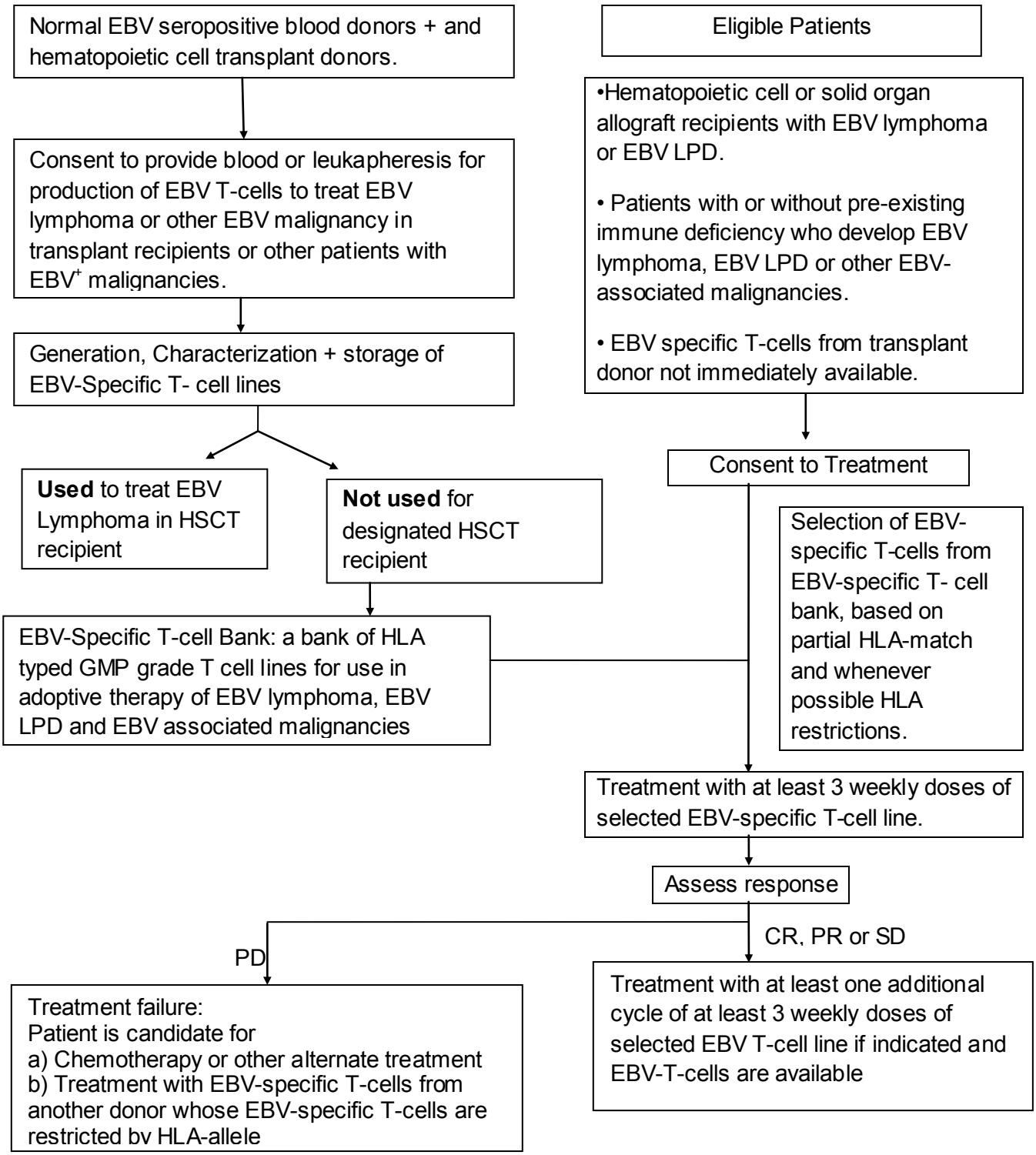
Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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

Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a single institution phase II trial designed to assess the therapeutic activity of Epstein-Barr Virus Immune T-lymphocyte lines derived from consenting EBV seropositive, partially HLA-matched third party donors. These T cell lines are generated, maintained, and cryopreserved in an MSKCC Transplant Program-administered cell bank for the treatment of EBV^+ lymphomas, EBV lymphoproliferative disease and other EBV-Associated Malignancies.



2.0 OBJECTIVES AND SCIENTIFIC AIMS

PRIMARY

1) To evaluate, in a Phase II single dose level trial, the therapeutic potential of adoptive immunotherapy with EBV-specific T-cells derived from HLA partially matched third-party donors in the treatment of EBV-induced lymphomas and EBV-associated malignancies including EBV^+ Hodgkin's and Non-Hodgkin's disease, EBV^+ nasopharyngeal carcinoma, EBV^+ hemophagocytic lymphohistiocytosis, and EBV^+ leiomyosarcoma.

2) To establish a centralized bank of cryoperserved, GMP (Good Manurfacturing Practice) grade, EBV-specific T-cell lines from consenting donors of defined HLA type and HLA restriction which can serve as an immediately accessible source of HLA partially matched EBV-specific T-cells for adoptive therapy of EBV lymphomas, EBV lymphoproliferative disease and other EBV-associated malignancies.

3) To estimate the overall survival, disease free survival, and probability of EBV relapse over time of patients who receive EBV-specific T-cells derived from HLA partially matched third-party donors.

SECONDARY

1) To evaluate the in vivo expansion and duration of engraftment of successive doses of transferred EBV-reactive lymphocytes within treated patients and to correlate these findings with the diseased patient's T-cell populations and general immune function.

2) To determine the incidence, kinetics and durability of pathological and/or clinical responses of EBV-induced malignancies to treatment with infusions of EBV-specific T-cells derived from histocompatible or partially HLA-matched EBV-seropositive normal third-party donors.

3.0 BACKGROUND AND RATIONALE

Epstein-Barr virus is a member of the herpes virus family of DNA viruses. In normal individuals, primary infection occurs in early adolescents and initially involves the parotid and salivary glands giving rise to either an asymptomatic infection or infectious mononucleosis (1). Following initial production of lytic virus, the virus infects and becomes latent in B-lymphocytes and remains so for the life of the individual. Infection of the B-lymphocytes results in their transformation and immortalization. However, during latent infection, only a limited number of viral genes are expressed. These include the nuclear antigens EBNA1-5 and the latent membrane proteins, LMP1 and LMP2 (2, 3). In normal seropositive individuals, 30-100/million circulating B-cells are transformed latently infected B-cells (4). The immune response to EBV includes both the generation of antibodies and the production of antigen-specific T-cells. However, control of latent infection depends upon the integrity of T-cell mediated immunity (5). Data from our own (6, 7) and other studies indicate that the principal effectors controlling EBV are HLA-class I-restricted $CD8^+$ T-cells specific for latent EBV antigens, particularly EBNA-3 (8, 9). EBV-specific HLA class II-restricted $CD4^+$ T-cells stimulate the $CD8^+$ T-cell populations but their role in controlling or eliminating EBV transformed cells is still controversial (10). In a normal seropositive adult, 0.5-5% of the circulating T-cells can be shown to be EBV-specific by analyses of $CD8^+$T-cells binding EBV peptide/HLA tetramer complexes.(11) Similarly, the frequencies of EBV reactive interferon-gamma positive T-cells and cytotoxic T-cell precursors range from $1/10^2$-10^3 and $1/10^3$-10^4 respectively (12).

In immunocompromised, T-cell deficient human hosts, EBV may cause either lymphoproliferative disorders or outright lymphomas (13). In addition, EBV latent infection has been implicated in the pathogenesis of up to 30 % of patients with Hodgkin's disease (14), a high proportion of nasopharyngeal carcinomas developing in the Orient (15) and leiomyosarcomas developing in patients with advanced AIDS, genetic immunodeficiencies or states of immunosuppression resulting from drugs used to prevent rejection or GVHD following organ or hematopoietic stem cell transplants (16).

EBV-LPD are particularly common among organ allograft recipients. Among renal allograft recipients their incidence ranges from 0.6 - 1% (17, 18). However, in more heavily immunosuppressed liver and heart allograft recipients, the incidences are 5% and 10-13% respectively (19, 20). The EBV-LPD developing in these patients are most commonly polyclonal (21). Such polyclonal disorders may regress spontaneously if treatment with immunosuppressive drugs is curtailed (21, 22). Alternatively, treatment with interferon-, acyclovir or B-cell specific monoclonal antibodies may induce durable remissions in a proportion of cases refractory to this approach (23, 24, 25). However, for patients developing monoclonal EBV lymphomas, none of these approaches has been consistently effective. Furthermore, despite chemotherapy, the long term survival has been estimated at 25-60% (23).

Recently, EBV-induced lymphomas have emerged as a significant complication of HLA non-identical related and matched related marrow allografts particularly when administered after selective depletion of T-cells (26, 27,32) or application of more intensive, T-cell targeted, cytoreductive regimens to ensure engraftment or prevent GvHD (28). These EBV-LPD resemble the EBV^+ lymphomas in AIDS patients and differ from the benign B-cell hyperplasias and polyclonal lymphoproliferative disorders observed in most renal and cardiac allograft recipients in that they most often present as malignant high-grade diffuse large cell B-cell lymphomas which are monoclonal (29). Their clinical course is fulminant. Indeed, studies have demonstrated that the median survival for patients afflicted with EBV lymphomas emerging early after hematopoietic stem cell transplants is only 31 days (30). Like EBV^+ lymphomas which emerge in the context of profound cell mediated immune deficits, these lymphomas express not only EBNA1 but also EBNA2, EBNA3, 4, 5 and LMP1 and 2. However, a striking feature of EBV lymphomas emerging in marrow allograft recipients is that they are almost invariably of marrow donor rather than host origin (26, 31). Furthermore, they develop only in the interval between completion of pre transplant immunoblative therapy and the reconstitution of functional donor T-cell derived immunity, which is usually 6-8 months post transplant (32). These malignancies thus reflect the rapid emergence of EBV transformed B-cell clones from the marrow graft itself if not controlled by EBV-specific T-cells.

Historically, treatment of systemic EBV lymphomas developing in patients with genetic immune deficiency, AIDS or organ grafts requiring chronic immunosuppression has entailed the use of combination chemotherapies similar to those used to treat non-Hodgkin's lymphoma, such as CHOP, PROMACE-CYTOBOM or m-BACOD. However, while initial complete remissions have been recorded in 20-72% of such patients, these remissions have been invariably short-lived (33-37). Furthermore, intensive chemotherapy has been complicated by a high mortality ascribable to opportunistic infections. As a consequence, median duration of survival post diagnosis of EBV lymphomas has ranged from 3-15 months. (33-37). For AIDS patients with EBV lymphomas of the central nervous system, which are consistently EBV^+, median survival duration is only 2-3 months (38,39).

More recently, the CD20 specific monoclonal antibody, Rituxan, has been employed to treat both EBV lymphoproliferative disorders and EBV lymphomas occurring in patients with genetic or acquired immunodeficiencies and in organ or hematopoietic stem cell transplant recipients. Rituxan used alone has induced initial responses in up to 70% of organ allograft recipients and up to 50 % of patients developing lymphomas following hematopoietic cell transplants (40, 41). However, responses overall have been sustained in no more than 50 % of patients (42). In those who relapse or fail to respond to Rituximab, these lymphomas have been consistently lethal.

The importance of T-cell immunity to the control of EBV infection had initially been suggested by the studies of Moss et al in the early 1970s (43). However, it was not until 1994 that the therapeutic potential of T-cells in the treatment of EBV lymphomas was formally demonstrated, when, our group reported a series of 5 patients who developed monoclonal EBV lymphomas following T-cell depleted allogeneic hematopoietic cell transplants who achieved durable remissions of pathologically documented monoclonal EBV lymphomas following infusions of small numbers of donor lymphocytes (26). In a subsequent study, we demonstrated that doses providing as few as 1,000 EBV-specific CTLPs could induce durable and complete remissions of widespread disease (32). Groups in the United States and Europe have subsequently confirmed these observations (44, 45). Furthermore, Rooney et al demonstrated that infusions of EBV-specific T-cells propagated in vitro prevented the development of EBV lymphomas in a large cadre of patients at risk who had received T-cell depleted hematopoietic stem cell transplants (46). In addition, our own group, the group at Baylor and a group in Milan have each demonstrated the capacity of such EBV-specific T-cells to induce complete regressions of EBV lymphomas without risk of subsequent development of graft versus host disease (47-49).

EBV specific T cells can also be effective in the treatment of other EBV associated malignancies. For example, recent clinical trials have demonstrated complete response rates in up to 20 % of patients with EBV^+ Hodgkin's disease who have received infusions of ex vivo propagated EBV-specific autologous T-cells (50). Similarly, a significant proportion of patients with EBV^+ nasopharyngeal carcinoma have also responded to infusions of either unselected allogeneic HLA-matched T-cells or in vitro propagated EBV-specific autologous T-cells (51, 69).

Our current, ongoing study (95-024) is a phase I/II clinical trial evaluating the toxicity and therapeutic potential of EBV-specific T-cells generated from a hematopoietic stem cell transplant (HSCT) donor in the treatment of EBV^+ lymphomas developing in recipients of such transplants. This study initially concentrated on evaluation of EBV-specific T-cells generated from HLA disparate unrelated and related hematopoietic cell transplant donors. We have focused on these donor/recipient pairs for 2 reasons: 1) These patients are at high risk of developing an EBV lymphoma, 2) These patients are also at the highest risk of developing severe acute and/or chronic graft vs. host disease if they receive unselected donor lymphocytes since these populations include significant frequencies of alloreactive T-cells capable of generating cytotoxic responses against major (HLA) as well as minor alloantigens. In Phase I of Protocol 95-024, a total of 18 marrow allograft recipients have received infusions of EBV-specific T-cells as treatment for documented monoclonal EBV lymphomas. Of the 18 HSCT recipients, 11 achieved complete and durable remissions of their EBV lymphomas following infusions of donor derived EBV-specific T-cells. No patient has developed acute or chronic GVHD.

Subsequent to the initial 18 HSCT patients treated, 95-024 underwent an IRB/FDA approved expansion to include EBV CTL infusions from partially HLA-matched seropositive third party donors.

Since that time, ten patients, who have received HSCTs, have been treated with EBV CTLs from 3^{rd} party donors. All infusions were well tolerated without toxicity or adverse event ascribable to the T cell infusions. Furthermore, no patient has developed acute or chronic GVHD. Six (6) of these patients (including one who was treated on a single patient use IND according to 95-024) achieved durable complete remissions. The remaining 3 patients were treated with 3^{rd} party EBV CTLs late in the course of their EBV lymphoma, and died of progressive disease. We have recently reported two of these patients who developed monoclonal EBV lymphomas following umbilical cord blood transplants which were successfully treated by sequential infusions of EBV specific T cells from an HLA-partially matched, consenting unrelated third party donor (70).

The HSCT cohort of 95-024 (Group 1) remains open as a phase II study, accruing patients to an extension of its Phase I arm.

In addition to HSCT recipients (Group 1), 95-024 is also designed to assess the toxicity and anti-tumor activity of HLA-haplotype matched EBV-specific T-cells in patients who develop EBV

lymphoma as a complication of the chronic immune suppression required to sustain an organ allograft or complicating AIDS (Group 2). To date, 7 organ allograft recipients and one patient with AIDS with EBV lymphomas were treated with either ex vivo expanded autologous (N=1), haplotype matched, related (N=5) EBV specific T-cells, or partially HLA-matched seropositive third party donors (N=2). Of 7 organ allograft patients, 1 patient received EBV CTLs late in the course of his EBV LPD and died of progressive disease, 1 patient is in durable CR, 2 patients achieved durable partial responses (>3 years), and the remaining 2 are with stable disease for >2 years post treatment.

The AIDS/organ allograft cohort of 95-024 (Group 2) remains open, and is accruing patients to its Phase I arm.

Our preliminary successes with third party donor-derived EBV specific T cells are consistent with the findings of Haque et al (52, 53), who used pre-generated EBV- specific T-cells derived from normal partially HLA-matched seropositive third party donors for cellular immunotherapy of EBV^+ lymphoproliferative disease in organ allograft recipients. In a phase II, multicenter trial conducted in Europe, HLA-partially matched EBV-specific T-cells were selected from a limited cryopreserved bank of EBV-specific predominantly CD8+ T-cell lines previously generated from normal EBV seropositive donors established by Crawford et al (54). The T-cells used for each patient were selected on the basis of partial HLA matching with the patient, EBV- specificity, and lack of reactivity against autologous or allogenic PHA-stimulated PHA blasts. A total of 33 transplant recipients were treated with an EBV lymphoproliferative disease that had progressed after discontinuation of immunosuppressive drugs. While these EBV LPD were not characterized as to clonality, they did include 13 EBVLPD which were refractory to Rituxan and/ or chemotherapy. There were 2 recipients of allogenic hematopoietic stem cell transplant and 31 organ allograft recipients. Treatment consisted of weekly infusions of 2×10^6 EBV-reactive T-cells/kg x 4. In each case, the infusions were well tolerated without acute toxicities. Furthermore, no patient experienced either an allograft rejection response or, in the marrow transplant recipients, GVHD. At 5-week post infusion, 64% had a significant response (12CR, 9 PR). At 6 months, these responses were sustained in all 12 patients with CR and in 5/9 patients who achieved a PR, or 52% of the series. Only 5 patients were monitored for engraftment of the T-cells; they were detected for 7 days in only one patient.

The use of partially HLA-matched seropositive third party donors in protocol 95-024, can obviate two central limitations to the broad application of EBV- specific T-cells for the adoptive immunotherapy of EBV-associated malignancies: the logistical difficulties in obtaining needed blood samples from donors and the prohibitively long time required to generate and isolate suitably EBV-specific T-cells in the numbers necessary for effective treatment. Without the use of third party cell lines, protocols rely on EBV- transformed B cell lines generated from the blood of the T-cell donor for in vitro immunization of the T- cells. Transformation of the B cells with the laboratory strain of EBV, B95.8 is very efficient. However, it takes 28-35 days before the transformed B cell line is sufficiently established and expanded to the numbers required for T-cell sensitization. Thereafter, an additional 30 days is required to culture and expand T- cells that are adequately EBV-specific and cytotoxic against EBV- transformed cells. This period of culture is also required to deplete the T cell lines of alloreactive T-cells that could cause either GVHD in marrow transplant recipients or rejection in an organ allograft recipient. Since the total time required to generate such T-cells (60-65 days) exceeds the median survival of patients with such lymphomas (31 days) (30), the EBV-specific T-cells need to be generated before the onset of lymphoma if they are to be consistently effective. For patients, other than a transplant recipient, who develop other EBV associated malignancies, generation of autologous EBV- reactive T-cells has been attempted, but often fails. Furthermore, EBV- specific T-cells generated from these tumor bearing hosts often fail to react against the limited spectrum of EBV antigens (LMP-1, LMP-2, EBNA-1) expressed by their tumors.

In the course of our study, we have, whenever possible, attempted to generate EBV-specific T-cells for patients at risk for this complication prior to or at the time of transplant. As a result, over 300 EBV-specific T-cell lines have been generated, characterized and cryopreserved for an individual

patient's use to treat an EBV lymphoma were it to develop. Fortunately, the incidence of EBV lymphoma in this patient group has been low (2-6%), and almost all of these patients have, by now, achieved reconstitution of functional T-cell immunity so that they are no longer at risk for developing an EBV lymphoma. As a result, we now have a significant number of HLA-typed, well characterized. HLA-restricted EBV-specific T-cell lines, generated under GMP conditions, which could be used for adoptive therapy of EBV lymphomas expressing shared HLA alleles, if the specific donors from whom they were generated provided second consent for their use in patients other than the specific individuals for whom they were transplant donors.

Based on the studies of Haque et al (52, 53) and our own initial experiences with third party EBV-specific T-cells, we propose 1) to test the clinical efficacy of such third party-derived EBV-specific T-cells, when adoptively transferred for the treatment of EBV-associated malignancies, as measured by clinical and radiologic responses. 2) To continue growing a bank of cryopreserved HLA-typed EBV-specific T-cell lines generated under GMP conditions from the blood of consenting donors as a source of third party derived, HLA partially matched EBV-specific T-cells of desired HLA restriction for adoptive therapy.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a non-randomized, single institution phase II single dose trial, designed to evaluate the therapeutic activity of HLA partially matched, EBV-specific T-cell lines generated in vitro from normal EBV-seropositive third party donors when adoptively transferred into patients afflicted with an EBV lymphoma, an EBV^+ lymphoproliferative disorder or another EBV-associated malignancy.

Patients eligible for this trial will include:

1) Recipients of allogenic hematopoietic cell (HSCT) transplants who develop an EBV lymphoma or EBV lymphoproliferative disease post transplant or who have rising levels of EBV DNA in the circulation following prior treatment of the EBVLPD with chemotherapy and/or Rituxan indicating that they are at high risk of developing a recurrent EBV lymphoma for whom EBV-specific T-cells from their HCT donor are not available

2) Recipients of organ allografts who develop a biopsy-proven EBV^+ lymphoma or lymphoproliferative disorder or recipients of organ allografts previously treated with chemotherapy and/or Rituxan who have persistent or recurrent elevations in levels of EBVDNA in peripheral blood mononuclear cells exceeding 500 copies/ml and are therefore at high risk for developing relapse of an EBVLPD

3) Any immunocompromised or genetically immunodeficient patient who develops a biopsy proven EBV lymphoma or lymphoproliferative disease or

4) Any patient afflicted with pathologically documented other EBV-associated malignancy (e.g. EBV^+ Hodgkin's and Non-Hodgkin's disease, EBV+ nasopharyngeal carcinoma, EBV^+ hemophagocytic lymphohistiocytosis or EBV^+ leiomyosarcoma).

The T cells to be used for adoptive therapy will be derived from T cell lines generated under GMP conditions from normal consenting EBV seropositive donors of defined HLA type. These T cell lines will be generated by repeated in vitro sensitizations with irradiated autologous B cells transformed

with the laboratory strain of EBV B95.8, and expansion in culture for at least 21-28 days. After testing to insure EBV-specificity, absence of alloreactivity, absence of endotoxin and microbial sterility, these T cells will be cryopreserved and maintained in a designated EBV-specific T-cell bank for subsequent use. Selection of an EBV-specific T-cell line from this bank for the treatment of a patient consenting to enter this phase II trial will be based on: 1) the presence of HLA alleles on the T cell line that are also expressed by the patient (and/or, in the case of HSCT recipients, the transplant donor because EBV lymphomas in such patients are usually of donor origin) 2) the availability of EBV-specific T cells in numbers adequate for treatment of the patient at the doses required and 3) ascertainment, whenever possible that the EBV-specific T-cell line is restricted by at least one HLA shared by the patient (and/or in the case of allogeneic HSCT recipients, the transplant donor).

4.2 Intervention

For each course of therapy, the EBV-specific T-cells will be administered at a dose of $2x10^6$ T-cells/kg recipient weight once weekly (+/- 3 days) for three doses. Each dose of T-cells will be infused intravenously over a period of 5 minutes. Patients who achieve complete remission over the three weeks of treatment may require only one course of the T-cells. Patients who have not experienced incremental grade III-IV toxicity following T-cell infusion who have not achieved a CR may receive additional courses of 3 weekly T cell infusions, each followed by 3 week periods of observation.

Because of the potential of EBVLPD and EBV-associated malignancies to progress rapidly, patients who continue to show progression following the 3 week observation period will be considered for alternative therapy (e.g. chemotherapy) or continued treatment with EBV-specific T-cells. In some cases, patients with aggressive EBV in the absence of a grade 3-4 SAE related to treatment with cells may be treated with an additional cycle of cells as early as one week post completion of T-cell therapy.

If the planned dose of EBV peptide sensitized T-cells ($2x10^6$ T-cells/kg) is not available, but the cells generated exhibit required levels of EBV specific cytotoxic activity and meet all other release criteria, patients may be treated at a dose level of 1x10^6 EBV specific T cells/kg. A +/- 20% variability of total cell dose is allowed.

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

If there are insufficient cells and no other viable options, patients may be infused with all available cells in two doses or with three doses. If three doses are given each dose should be as close to 1 x 10^6 T-cells/Kg as possible.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Donors of EBV-Specific T-cells

The EBV-specific T-cell lines to be used for treatment in this protocol and to be maintained cryopreserved under GMP conditions in the Adoptive Immune Cell Therapy Facility at MSKCC, will be derived from the 3 groups listed below:

1) Normal, HLA-typed, EBV-seropositive donors of allogeneic hematopoietic progenitor cell or organ allografts, who previously consented and gave blood or leukocytes to establish EBV-specific T-cells to be used to treat an EBV lymphoma were it to develop in the patient to whom they donated a hematopoietic cell graft or organ. Once that patient has reconstituted immune function and is no longer at risk of an EBV lymphoma, the donors will be informed of the purposes of this T-cell bank and will be asked for consent, in writing, to the addition of their EBV-specific T-cell line generated from their blood to the bank and to the use of these T-cells to treat an EBV lymphoma or EBV-associated malignancy in a patient other than the patient for whom they were originally intended. This group of previously established EBV-specific T-cell lines currently constitutes a bank of approximately 300 separate donor-derived lines.
2) Normal, HLA typed, EBV-seropositive related and unrelated hematopoietic cell transplant donors who, from now on, are requested to consent to provide blood or blood white cells to make EBV-specific T-cells for treatment or prevention of an EBV-lymphoma in the recipient of their transplant will also be asked whether the EBV-specific T cell line generated can be transferred to the bank and used for another patient, at such time as the transplant recipient is no longer at risk for this complication.
3) Normal, HLA typed, EBV-seropositive volunteer blood donors whose T cells would add to the HLA diversity of the T cells available in the Immune Cell Therapy Bank.

Adequate health for donation is determined by institutional (related donor) or NMDP (unrelated donor) guidelines. Normal donors are evaluated for evidence of prior sensitization to EBV by EBV serology. They are also typed for HLA-A,B, C and DR.

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

Because of the need to, at least, partially match the donor tissue type with the patient's tumor, our goal is to have many 3rd party EBV CTL lines available. We estimate that a bank containing EBV-immune T cells from about 300-500 donors of different types would be able to supply partially matched T cells for over 90% of patients who have EBV disease.

Volunteer blood donors in Group 3 will be requested to provide the second of the two blood samplings listed below. The first sample may be provided, as well, if clinically feasible. Donors in Groups 1 and 2 will have already provided the two donations listed below upon initial consent to the T cell infusion protocol (IRB # 05-065, 07-055, 95-024, or 12-086) for the primary recipients of their allogeneic hematopoietic progenitor cell or organ allografts.

i) An initial donation of 25 ml of heparinized blood-this blood is used to establish a B cell line transformed with the B95.8 laboratory strain of EBV, which is required for subsequent in vitro sensitization of the donor's T-cells against EBV. The establishment and testing of an EBV transformed autologous B cell line suitable for T-cell stimulation requires 4-5 weeks of in vitro culture.

ii) A donation of a single standard 2 blood volume leukapheresis collected in standard ACD anticoagulant. If it is impossible to collect a leukapheresis from some of the donors, a unit of whole blood will be acceptable. However, the AICTF (Adoptive Immune Cell Therapy Facility manufacturing the clinical grade cell products under GMP conditions at MSKCC) may only be able to generate a limited number of T cells from a unit of blood. This blood is required for isolation of the T-cells to be sensitized with the donor's EBV B cell line and propagated in vitro. In addition, it is required to

provide autologous feeder cells essential to sustain T- cell growth without the risk of stimulating the growth of alloreactive T-cells capable of inducing GVHD.

In addition to providing written consent to these donations of blood for the purpose of generating EBV- specific T-cells for potential use in the treatment of an EBV lymphoma, volunteer donors in Groups 2 and 3 will be informed of the following potential applications of the blood cells donated:

The use of a fraction of the cells isolated to generate:

a) immune T-cells specific for another virus, called cytomegalovirus, that can cause lethal infections in transplant recipients

Such T-cells could be used, under separate protocols to treat CMV infection (IRB # 12-086).

Inclusion of the use of blood cells for the purpose of also generating T cells against CMV can obviate the need for an additional leukapheresis or unit of blood for this purpose. T-cells will be stored and maintained cryopreserved under GMP conditions in the Adoptive Immune Cell Therapy Facility at MSKCC, to be used for the treatment of patients in whom these cells may be therapeutic.

5.2 Generation of EBV-Specific T-Cells From the Blood of Selected Donors

For the generation of EBV-specific T-cells, 25 ml of heparinized blood is initially drawn and used to prepare an EBV transformed B-cell line. To prepare these lines, Ficoll-Hypaque separated mononuclear cells are washed and exposed to filtered bacterial, fungal and mycoplasma-free supernatants of the EBV secreting B95-8 marmoset cell line. The B95-8 cell line used is negative for retroviruses and other animal viruses. After exposure, the cell lines are propagated in RPM1-1640 with glutamine and 10% pooled, screened fetal calf serum. After transformation of the B cells by EBV, the EBV transformed cell line is expanded in the same medium together with acyclovir to prevent generation of infectious EBV. Theses cell lines can then be used as a stimulus for the generation of T-cells from the same donor. Prior to use as stimulators, the EBV transformed cells are also irradiated to 9,000 rad to eradicate the capacity of these lines to grow within the co-cultures used to propagate EBV-specific T-cells. A calibrated, certified blood irradiator will be used for irradiation of these targets.

EBV-specific T-cell lines will be generated from cultures of purified peripheral blood T-lymphocytes sensitized with the EBV transformed B-cells. Effector T cells can be generated from 50 cc of blood from an EBV-seropositive donor. However, in order to have adequate numbers of irradiated autologous mononuclear cells as feeder cells during the propagation of the EBV-specific T cells, a standard 2.0 blood volume leukapheresis or a unit of blood obtained from the donor is required. Peripheral blood mononuclear cells will be separated from the leukapheresed cells by density gradient centrifugation on Ficoll-Hypaque. An aliquot of these cells will be depleted of monocytes by incubation on plastic surfaces to remove adherent cells (or by immunoadsorption with CD14 microbeads if frozen/thawed sample is used, so as to prevent clumping and loss of the cells during the depletion procedure). T-cells will then be enriched and purified by removal of NK cells and B-cells with magnetic beads coupled with CD56 and CD19 clinical grade microbeads. Purified peripheral blood T-lymphocytes ($1x10^6$ cells/ml) will then be stimulated with $5x10^4$ irradiated (9,000r) autologous EBV transformed B-cells. The T-cell: EBV+ BLCL ratio is therefore 20:1, a condition which favors development of EBV-reactive T-cells. These cells are propagated without added cytokine for 10 days, and thereafter, expanded by growth in medium supplemented with clinical grade recombinant IL-2 (concentration 10 units/ml) and IL-15 (concentration 10ng/ml). Feedings with medium and IL-2 are administered at 3 day intervals. IL15 is added weekly.

The activity of the anti-EBV specific T-cells will be assayed at ~28-42 days of culture as soon as the number of cells sufficient for the treatment dose is obtained. Included in these assays will be analyses of the cytolytic activity of the T-cells against the autologous BLCL and against autologous donor and allogeneic host derived PHA blasts. After in vitro propagation for 28-42 days, populations of T cells exhibiting cytolytic activity against autologous EBV B cell lines and appropriate specificity

will be selected for use as effectors for adoptive immunotherapy. The criteria for selection of effector cell populations appropriate for adoptive immunotherapy will include:

1) The EBV-specific T cell populations must be culture negative for bacteria, fungi, mycoplasma and must be tested and shown to contain ≤ 5 EU/ml cell dose of endotoxin.

2) The EBV-specific T cell populations must exhibit high levels of specific lysis of EBV-transformed autologous B cell lines (>25% specific lysis at effector target ratios of 25:1), but not against autologous PHA blasts (<10%) in standard ^{51}Cr release assays.

3) The EBV-specific T cells must not exhibit significant cytotoxicity against the patient's PHA blasts or against HLA-mismatched EBV transformed B cell lines in standard 51Cr release assays.

5.3 Cryopreservation and Storage

Prior to use, these EBV- specific T-cells may be cryopreserved at a concentration of up to 30 x 10^6 cells/ml in 10% DMSO, 16% human serum albumin and normal saline for intravenous infusion in cryovials labeled with the unique identifier number and unique bar code of the donor, name of the initially intended transplant recipient, MSKCC MRN of the recipient, component AICT#, component name, date the component was started, date the component was frozen and the number of cells frozen per container. Cryovials will be stored in the vapor phase of a monitored liquid nitrogen freezer designated for this purpose in the AICT cell manufacture facility at MSKCC.

5.4 A Central Bank of Cryopreserved EBV- Specific T-cells for Adoptive Cell Therapy

The MSKCC Bank of Cryopreserved Immune T-cells for Adoptive cell Therapy will be maintained in monitored liquid nitrogen freezers designated for this purpose in the AICT Cell Manufacture Facility at MSKCC. Each T-cell line accrued to this Bank will be logged in a computerized registry recording the donor providing the T-cells, the donor's eligibility, the donors written consent, the donor's unique AICT number and the Q/A testing specifications described above under Generation of EBV- specific T-cells. Each sample will be labelled as described above under Cryopreservation and Storage.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

6.1.1 Subject Inclusion Criteria

- Pathologically documented EBV antigen positive lymphoproliferative disease, lymphoma or other EBV-associated malignancy.

OR

- Evaluable disease as demonstrated by clinical and/or radiologic studies with current or prior elevated blood levels of EBVDNA exceeding 500 copies/ml by quantitative real time pcr.

OR

- Persistent or recurrent elevations in levels of EBVDNA exceeding 500 copies/ml in patients previously treated for EBVLPD with chemotherapy and/or Rituxan who do not yet have clinically or radiologically evaluable disease but are at high risk of disease recurrence.
- EBV-specific T-cells from donor of the patient's transplant are not available.
- EBV-specific T-cells are available for adoptive immune cell therapy from a consenting third party donor. The third party EBV CTLs to be administered will be selected on the basis of two criteria: 1) that they are matched for at least 2 HLA antigens and 2) that they are

restricted by an allele shared with the EBV$^+$ malignancy (if known), or with the donor in HSCT recipients, or patient in organ transplant or immunodeficient patients.

- KPS or Lansky score ≥ 20.
- A life expectancy of at least 6 weeks.
- Adequate bone marrow, heart, lung, liver and kidney function at the time treatment with EBV-specific T-cells is initiated, including:
    a) Absolute neutrophil count (ANC) ≥ 1K/mcL, with or without GCSF support
    b) Platelets ≥ 20K/mcL
    c) Creatinine ≤ 2.0mg/dl
    d) ALT, AST < 3.0x and total bilirubin < 2.5x the institutional ULN
    e) Stable blood pressure and circulation not requiring pressor support
    f) Adequate cardiac function as demonstrated by EKG and/or echocardiographic evidence (may be performed within 30 days prior to treatment)

  However, abnormalities of specific organs will not be considered grounds for exclusion if they are the result of the EBV$^+$ malignancy or its treatment (e.g. a renal allograft recipient with an EBVLPD may be on dialysis because the allograft was rejected when the immune suppression was stopped as a first approach to treatment of the EBVLPD). At the discretion of the investigator, patients with elevated but stable creatinine will not be precluded from treatment on study.
- There is no age restriction to eligibility for this protocol.

It is expected that five types of patients afflicted with EBV-associated lymphomas, lymphoproliferative diseases or malignancies will be referred and will consent to participate in this trial. These are:

1) Patients developing EBV lymphomas or lymphoproliferative disorders following an allogeneic hematopoietic progenitor stem cell transplant (HSCT) (ie: marrow, PBSC, or umbilical cord blood). In these cases, the HSCT donor, if EBV-seropositive, will be used as the donor of EBV-specific T-cells for adoptive immunotherapy wherever possible, because the EBV-LPD are almost invariably derived from that marrow donor. These patients will be enrolled onto protocol # IRB 95-024. However, if the HSCT donor is EBV seronegative or not readily available (e.g. a cord blood transplant), the patient will be a candidate to receive EBV- specific T-cells generated from a third party seropositive donor that have been generated and stored in the MSKCC bank of cryopreserved immune T-cells for adoptive cell therapy. For these patients, the third party donor derived T cells to be used will be selected primarily on the basis of 1) matching for, at least, 2 HLA antigens and 2) one restricted allele shared by the transplant donor and recipient. However, priority is given to T cells partially HLA antigen matched with, and restricted by, HLA alleles of the transplant donor, since EBV + lymphomas in HSCT recipients are usually (but not always) derived from the transplant donors' cells.

2) Patients developing EBV lymphomas or lymphoproliferative disorders following an allogeneic organ transplant. In these cases, the lymphoma is usually of recipient origin. EBV-specific T-cells will be selected from the MSKCC Bank expanded from an EBV-seropositive normal donor who is at least matched for 1) 2 HLA antigens and 2) one restricted allele with the EBV lymphoma. If the origin of the lymphoma is unknown, T-cells partially matched with the transplant recipient will be used, since these lymphomas are usually of host origin. Using this approach to donor selection, it is expected that the EBV-specific, HLA restricted cytotoxic T-cells expanded from the HLA partially-matched donor would be able to recognize and kill lymphoma cells presenting EBV antigens in the context of an appropriate HLA restricting element. Priority will be given to the use of partially

matched EBV specific T cells known to be restricted by an HLA allele shared by the lymphoma (or, if unknown, the patient).

3) Patients with AIDS developing EBV lymphomas or lymphoproliferative diseases as a consequence of the profound acquired immunodeficiency induced by HIV. For such patients, EBV specific T-cells from third party seropositive donors who are HLA compatible in 1) at least 2 HLA antigens and 2) one restricted allele shared by the patient will be used. Selection of T cells known to be restricted by an HLA allele shared by the patient will be given priority.

4) Patients who develop EBV lymphomas or lymphoproliferative diseases or other EBV-associated malignancy as a consequence of profound immunodeficiencies associated with a congenital immune deficit or acquired as a sequela of anti-neoplastic or immunosuppressive therapy. For these patients, normal, EBV specific T-cells from third party seropositive donors who are HLA compatible in 1) at least 2 HLA antigens and 2) one restricted allele shared by the patient will be used. Again, selection of T cells known to be restricted by an HLA allele shared by the patient will be given priority.

5) Patients who develop other EBV-associated malignancies without pre-existing immune deficiency, including: EBV^+ Hodgkin's and Non-Hodgkin's disease, EBV^+ nasopharyngeal carcinoma, EBV^+ hemophagocytic lymphohistiocytosis, or EBV^+ leiomyosarcoma. Normal, EBV specific T-cells from third party seropositive donors who are HLA compatible in 1) at least 2 HLA antigens and 2) one restricted allele shared by the patient will be used. Again, selection of T cells known to be restricted by an HLA allele shared by the patient will be given priority.

6.1.2 Donor Eligibility

Donors in Groups 1 and 2 (Section 5.1) would have already been determined to be eligible and will have donated blood or leukocytes to establish EBV-specific T-cells under IRB # 05-065, 07-055, 95-024, or 12-086. There are no additional eligibility requirements for these donors.

Donors in Group 3 (section 5.1), however, will need to meet the following eligibility requirements prior to donation:

a) Donors must satisfy the criteria specified in FDA 21 CFR 1271.

b) Donors must be typed for HLA-A, B, C and DR

c) Donors must have a hemoglobin value > 10g/dl

d) Donors must be capable of undergoing, at least, a single standard 2 blood volume leukapheresis or a donation of one unit of whole blood

6.2 Subject Exclusion Criteria

6.2.1 Patient Exclusion Criteria

The following patients will be excluded from this study:

a) Patients with active (grade 2-4) acute graft vs. host disease (GVHD), chronic GVHD or an overt autoimmune disease (e.g. hemolytic anemia) requiring high doses of glucocorticosteroid (>0.5 mg/kg/day prednisone or its equivalent) as treatment

b) Patients who are pregnant

c) Patients with severe comorbidities, not related to their EBV-associated malignancy, that would be expected to preclude their survival for the 6 weeks required to assess response of T cell therapy

d) Patients eligible for MSK protocol # 16-803 (EBV-CTL-201).

6.2.1 Donor Exclusion Criteria

a) HTLV/HIV(+) or Hepatitis B or C antigen(+) donors

b) Donors who are known EBV seronegative

7.0 RECRUITMENT PLAN

7.1 Patient Recruitment

Patients with EBV lymphoproliferative diseases, lymphomas or other EBV-associated malignancies will be referred from (but not limited to) the Allogeneic Marrow Transplant program at MSKCC, the marrow and organ transplant programs at other centers in New York, New Jersey and Connecticut and from transplant centers participating in the national BMT Clinical Trials Network. We are also establishing collaborations with the organ transplant programs at Weill-Cornell Medical Center, Columbia Presbyterian Hospital and Mount Sinai Hospital to refer patients that develop a PTLD to our center as potential candidates for this trial.

7.2 Donor Recruitment

Volunteer donors will be recruited from the Allogeneic Marrow Transplant program at MSKCC.

Other donors would have already been enrolled onto a previous MSKCC/NMDP IRB approved study (ie: MSKCC IRB # 95-024, 05-065, 07-055, and 12-086) and have already provided blood donations under those protocols. These donors will be asked to provide separate consent for transfer of their cells to the third party bank for potential use in patients enrolled onto this protocol. Because these donors were only under the care of MSKCC for the period in which they donated cells for their primary recipients and have since returned home, they will be mailed a copy of the consent form to review during the consent discussion, which will occur over the phone. If they agree to 3rd party use of their cells, the consent will be signed and sent back via MSKCC provided pre-paid/addressed envelope.

8.0 PRETREATMENT EVALUATION

8.1 Patient Pretreatment Evaluation

The following evaluations will be performed within approximately 30 business days prior to treatment on study, with the exception of EBV DNA and blood samples, which will be drawn within approximately 3 business days prior to beginning treatment. Certain tests may be held at the discretion of the treating physician:

- EBV DNA will be quantitated in peripheral blood mononuclear cells, using a quantitative pcr-amplified assay incorporating appropriate controls to define EBV DNA copy number/ml of blood.

- Radiographic analyses, if clinically indicated, including PET/CT scans and/or MRI scans to define extent of disease will be obtained and any lesions quantitated as to size by three-dimensional measurements.
- Biopsy of affected or suspicious tissue will be obtained when clinically feasible. For immunocompromised patients with significant levels of circulating EBV DNA, who are at high risk of an EBV lymphoma, no suspicious lesions may be detected or appropriate for biopsy. However, patients who have clinically overt EBV LPD, or other EBV associated malignancy, will usually have tumor or lymph nodes assessable for such biopsies. The purpose of these biopsies is to obtain viable tumor cells. These cells will be analyzed to determine the genetic origin of the EBV-LPD in transplant recipients (donor or host) by ascertainment of HLA type or definition of donor or host unique autosomal or sex chromatin polymorphisms by pcr-amplified RFLP analyses. They will also be evaluated for the clonality of the lymphoma as defined by evaluation of immunoglobulin VDJ rearrangements and the structure of EBV episomal DNA genomic termini. For patients with other EBV associated malignancies, the tumor cells can be analyzed for their expansion of EBV antigens and for EBV type by DNA sequence. Pathologically confirmed lymphomas will be characterized by histopathologic and immunohistochemical analysis for immunophenotype and expression of EBV-associated RNAs (EBERs) and proteins such as EBNA-1, EBNA-2 and/or LMP-1. Whenever possible, an aliquot of cells will be cryopreserved.
- Pregnancy Test for women of childbearing age

Patients will also have blood samples drawn to evaluate:

- General immune function. These studies will include quantitation of T- and B-cells and their subsets by immunophenotypic analysis and quantitation of the capacity of T-cells to respond to mitogens, antigens, and allogeneic cells.
- EBV-specific immunity. Peripheral blood mononuclear cells isolated from the patients will be evaluated for their capacity to proliferate and to generate cytotoxic T-cells specifically reactive against autologous EBV-transformed B-cells. In addition, limiting dilution analyses will be performed to ascertain the frequency of CTL precursors in the peripheral blood T-cell population which exhibit specific reactivity against EBV as well as the frequency of T-cells exhibiting a capacity to react against a third party allogeneic stimulus.

9.0 TREATMENT/INTERVENTION PLAN

Each patient consenting to participate in this Phase II trial will be treated with in vitro expanded EBV-specific T-cells. Patients will be initially stratified into two groups.
Group 1 will consist of:

A. Allogeneic marrow graft recipients who are severely immunocompromised and may have more prolonged or sustained T cell engraftment and, therefore, could be at risk for GvHD
B. Patients with severe congenital or antineoplastic drug induced immunodeficiency who could be durably engrafted and are therefore at risk for GvHD

Group 2 will consist of:

A. Organ allograft recipients. Although immunocompromised, these patients can reject third party blood cells. Thus, the risk of sustained engraftment or GvHD is low
B. AIDS patients who will almost invariably reject the infused T-cells and are therefore at very low risk of GvHD

C. Patients who develop other EBV-associated malignancies without pre-existing immune deficiency who are also expected to reject the infused T cells and are at low or no risk of GvHD

9.1 Treatment for Patients in Group 1

Patients in Group 1 will each receive a course of three weekly infusions of EBV-specific T-cells. Each weekly dose will provide 2 x10^6 T-cells/Kg recipient weight (+/- 3 days) from the donor's EBV-specific T-cell line. After the third dose, patients will be observed for approximately 3 weeks. If only grade 0-2 toxicity related to the infusion has been observed and GVHD has not developed or exacerbated, patients in CR, PR, or SD may be treated with a second course of 3 weekly infusions of EBV-specific T-cells. After the second 3 week course of cell infusions, responses will be graded if there is a change in response. If at that time, only 0-2 toxicity related to the infusion has been observed and GVHD has not developed or exacerbated, and no further progression of the EBV LPD or malignancy has been detected, these patients may be treated with additional infusions of EBV-specific T-cells at the same dose level. If, on the other hand, the patient has already achieved a CR of the EBV LPD or malignancy, the secondary infusions may be either administered or electively held or postponed. If at any time in the course of these infusions a grade 3-4 toxicity related to the infusion has been observed, no further doses of cells will be administered.

Because of the potential of EBV LPD and EBV-associated malignancies to progress rapidly, patients who continue to show progression following the 3 week observation period will be considered for alternative therapy (e.g. chemotherapy) or treatment with additional EBV-specific T cells. Patients may receive treatment from a different third party donor that is specific for EBV but restricted by a different HLA allele shared by the EBVLPD.

The rationale for using such EBV-CTL from a second HLA partially matched third party donor is based on our prior experience (72, 73) and that of Gottschalk et al (74) indicating that failures of adoptive therapy in the treatment of EBVLPD are usually due to failure of T-cells generated against EBV strain B95.8 to recognize the tumor cells transferred with the endogenous EBV in the patient. This failure is often caused by EBV virus mutations that result in deletions or alterations in the amino acid sequence of immunodominant peptide epitopes of an immunogenic latent protein like EBNA 3C targeted by the T-cells. Because T-cells restricted by a different HLA can be expected to be specific for a different, and potentially non-mutated epitope, adoptive transfer of such T-cells from a separate donor could provide an alternative and potentially effective approach to the treatment of EBVLPD transfused by a mutated EBV strain of this type.

In some cases, patients with aggressive EBV, in the absence of a grade 3-4 SAE related to treatment with cells, may be treated with an additional cycle of cells as early as one week post completion of T-cell therapy.

If the initial planned doses of EBV peptide sensitized T-cells (2 x10^6 T-cells/Kg) are not achieved or available, but the cells generated exhibit required levels of EBV specific cytotoxic activity and meet all other release criteria, patients may be treated at a dose level of 1 x10^6 T-cells/Kg recipient weight. A +/- 20% variability of total cell dose is allowed.

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

If there are insufficient cells and no other viable options, patients may be infused with all available cells in two doses or with three doses. If three doses are given each dose should be as close to 1 x 10^6 T-cells/Kg as possible.

9.2 Treatment for Patients in Group 2

Patients in Group 2 will also receive 3 weekly intravenous infusions of 2 x 10^6 EBV-specific T-cells/kg recipient weight (+/- 3 days). After the third dose, patients will be observed for at least 3 weeks. If only grade 0-2 toxicity related to the infusion has been observed and GVHD has not developed or exacerbated, patients in CR, PR, or SD may be treated with a second course of 3 weekly infusions of EBV-specific T-cells. After the second 3 week course of cell infusions, responses will be graded if there is a change in response. If at that time, only 0-2 toxicity related to the infusion has been observed and no further progression of the EBV LPD or malignancy has been detected, these patients may be treated with additional infusions of EBV-specific T-cells at the same dose level OR at a dose of 1 x 10^7 T-cells/kg/dose. If, on the other hand, the patient has already achieved a PR or CR of the EBV LPD or malignancy, the secondary infusions may be either administered or electively held or postponed. If at any time in the course of these infusions a grade 3-4 toxicity related to the infusion has been observed, no further doses of cells will be administered.

This five-fold increment in dose planned for the secondary courses, to be administered to patients in Group 2 is based on these considerations: 1) the expected low incidence of toxicity and complications of the T-cell infusions; 2) the expectations that patients in Group 2, once sensitized, will eliminate secondary doses of T-cells too rapidly to permit them to have an effect if the initial dose is again used. This reactivity against donor cells will also likely limit their effectiveness beyond two courses of therapy.

Because of the potential of EBV LPD and EBV-associated malignancies to progress rapidly, patients who continue to show progression following the 3 week observation period may be considered for alternative therapy (e.g. chemotherapy) or treatment with additional EBV-specific T cells. Patients may receive treatment from a different third party donor that is specific for EBV but restricted by a different HLA allele shared by the EBVLPD. In some cases, patients with aggressive EBV in the absence of a grade 3-4 SAE related to treatment with cells may be treated with an additional cycle of cells as early as one week post completion of T-cell therapy.

If the planned dose of EBV peptide sensitized T-cells (2 x10^6 T-cells/Kg and/or of 1 x 10^7 cells/kg) is not achieved, but the cells generated exhibit required levels of EBV specific cytotoxic activity and meet all other release criteria, patients may be treated at a dose level of 1 x10^6 T-cells/Kg recipient weight. A +/- 20% variability of total cell dose is allowed.

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

If there are insufficient cells and no other viable options, patients may be infused with all available cells in two doses or with three doses. If three doses are given each dose should be as close to 1 x 10^6 T-cells/Kg as possible.

Initial published studies and our own experience using third party, HLA partially matched virus-specific T-cells, generated in vitro, for adoptive immunotherapy or prophylaxis against CMV or EBV suggest that the toxicity associated with the T-cell infusions will be minimal (29, 46, 52, 53). Because of the in vitro selection for T-cells reactive against EBV, the frequency of alloreactive T-cells capable of inducing GvHD or, in organ allograft recipients, an organ rejection episode should also be extremely low. Nevertheless, the risks of fostering GvHD in marrow allograft recipients must be considered substantive, since these patients will already have been engrafted with an allogeneic donor-derived marrow and lymphoid progenitors. On the other hand, AIDS patients, organ graft recipients, and otherwise normal patients with other EBV associated malignancies, even in the extremes of virus or drug induced immunosuppression, retain the capacity to reject foreign hematopoietic cells and do not develop GvHD even when repeatedly transfused with unirradiated blood (39). Thus, these patients who constitute group 2would be expected to be at low risk for GvHD even if substantial numbers of alloreactive T-cells were to be transfused in the EBV-specific T-cell inoculum.

9.3. Use of Other Medications and Treatments During the Trial of EBV-Specific T-Cell Infusions

1) Graft Versus Host Disease Prophylaxis and Treatment

Following the infusions of third party donor-derived EBV-specific T-cells, no additional drug prophylaxis will be given to prevent GvHD. In patients who do develop GvHD, standard clinical and pathological criteria will be used to establish the diagnosis. GvHD will be graded according to BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al (66). A patient who develops significant (grade II or greater) GvHD following infusion of third party donor-derived EBV specific T cells will be considered for treatment with high-dose methylprednisolone. Refractory grade II or progressive grade III or IV GvHD will be treated according to separate protocols.

2) Concurrent Medications

a) Any non-cytotoxic, non-chemotherapeutic medications needed in the management of the patient or the prevention and treatment of bacterial, fungal or parasitic infection are allowed. Since Rituxan is being used to treat EBV+ B cell lymphomas, and most patients referred for treatment with EBV CTLs are those whose disease continues to progress despite Rituxan, ongoing treatment with Rituxan will also be permitted.

b) Marrow transplant recipients receiving, steroids, or antimetabolite drugs as immunosuppressive agents for prophylaxis against GvHD should, whenever possible, have these agents discontinued at least one week prior to initiation of treatment with EBV-specific T-cells. Patients requiring these agents as treatment for acute or chronic GvHD or as essential prophylaxis against organ graft rejection may continue maintenance therapy with cyclosporine, tacrolimus or sirolimus since data from our own and other centers suggest that these agents do not preclude the viability or antigen-specific cytotoxic function of pre-sensitized and expanded memory T-cells. However, treatment with systemic steroids, anti-thymocyte globulin, and/or Imuran should be discontinued whenever possible prior to initiation of T-cell infusions since theses agents will eliminate the infused T cells or severely compromise their capacity to grow in the treated patient. It is recognized, however, that clinical circumstances may mandate maintenance of treatment with steroids (e.g., GVH treatment in patients who, by virtue of kidney or liver toxicity, cannot tolerate cyclosporine, or patients to be treated for EBV lymphomas of the brain who may require steroids to reduce neurologic symptoms induced by edema surrounding the tumor). In such cases, doses will be maintained at as low a level as considered clinically safe and appropriate prior to initiation of infusions of EBV specific T cells.

c) Organ transplant recipients receiving immunosuppressive agents to prevent organ allograft rejection may continue maintenance therapy with cyclosporine, tacrolimus or sirolimus. Of these, sirolimus is preferred since this MTOR inhibitor may, itself, inhibit growth of EBV transferred cells (71). If possible, treatment with systemic steroids should be discontinued prior to initiation of T-cell

infusions. Anti-thymocyte globulin and/or Imuran should not be given. Since polyclonal EBV-lymphoproliferative diseases may regress following cessation of immunosuppression, cessation of agents other than cyclosporine, tacrolimus or sirolimus should ideally be completed two weeks prior to initiation of infusions of EBV-specific T-cells to permit assessment of the effect of altered immunosuppression on the progress of the EBV lymphomas to be treated. As for marrow graft recipients, clinical circumstances may preclude cessation of steroids. In such cases, doses should be reduced to as low a dose as is safe and appropriate during the T cell infusions and for 3 weeks after each course.

d) AIDS patients developing EBV lymphomas while being treated with AZT and other antiviral agents may continue to receive these agents at the same dose.

e) All patients developing EBV lymphomas while receiving antiviral agents such as acyclovir or ganciclovir for prophylaxis or treatment of herpes simplex, zoster or CMV infections may be maintained on these agents at the same dose during treatment with EBV-specific T-cells.

f) Blood support All blood products for transfusion, except the infusions of EBV-specific T-cells will be irradiated to inactivate lymphocytes capable of initiating lethal GvHD. Blood products are irradiated in the Blood Bank, using a cesium gamma emitter.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

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

- Patients will be evaluated clinically by vital signs pre, post, and at 1 and 2 hours following initiation of each infusion of EBV-specific T-cells
- Patients will receive interim history, review of systems, and physical exam prior to starting a cycle of EBV-specific T-cells. Assessments will then be performed weekly, as clinically indicated, for the first 8 weeks post-initial infusion with EBV-specific T-cells, then monthly until 1 year post initial infusion
- Patients will also be assessed for the effects of the adoptively transferred EBV-specific T-cells on circulating levels of EBV DNA. For these studies, EDTA blood (10cc) will be drawn on day 1, then weekly for the first 8 weeks post initial infusion, and monthly until 1 year post initial infusion
- In order to assess the effects of infusions of EBV-specific T-cells on the level of circulating virus-specific cytotoxic T-cells in the circulation, limiting dilution analyses quantitating EBV-specific CTLp will be performed using 10-20 cc blood samples drawn from the patient on days 1, 7, and 14 post initial infusion of EBV CTLs (ie: prior to the 2^{nd} and 3^{rd} T cell doses). EBV-specific CTLp will also be assessed on day 15, at weekly intervals for 6 weeks post-initial infusion, and at 3, 6, 9, and 12 months post-initial infusion
- Research samples may be collected at approximately day 0, day 7, and day 14 testing for cytokines. Additional samples will be collected if there are clinical indications to monitor for cytokines.
- CBC and Flow Cytometry 7 panel will be obtained on the same days EBV CTLp are drawn
- Evaluations of T-cell proliferative responses to mitogens and non-EBV microbial antigens will be drawn at 5 weeks post initial infusion of EBV CTLs

- PET/CT and/or MRI scans will be performed on patients with radiologically identifiable disease prior to T cell therapy at 5 weeks post initiation of each 3 week course of cells and at 6 and 12 months post initial infusion. Scans may be performed earlier if clinically indicated.

- In our experience, patients with Nasopharyngeal Carcinomas do not typically show response to treatment after one cycle. These patients may forgo scans at Week 5 and will have scans performed approximately 2 weeks post completion of a second cycle of cells. Evaluation for these patients will occur after a second cycle of cells, unless clinically indicated.

- Whenever possible and if clinically indicated, lymph nodes or parenchymal sites of lymphoma will be biopsied 2-3 weeks after the first and/or second 3 week course of EBV-specific T-cells to assess the effects of the infusions on the tissues involved. Biopsied tissues will be examined for histopathologic evidence of EBV+ tumor cells. Immunohistochemical analyses will also be performed to characterize cells infiltrating the biopsied tissues. If sufficient biopsy tissue is available, single cell suspensions will be derived from portions of involved tissue and characterized as to immunophenotype, expression of EBV antigens, origin (donor or host) and clonality. Origin will be determined by analysis of separated B- and T lymphocytes for donor or host unique X, Y and/or autosomal polymorphisms by FISH and/or pcr amplified RFLP analyses. Cells will also be analyzed for residual populations of EBV+ tumor cells bearing the distinctive VDJ rearrangement of the Ig gene and the distinctive homogenous genomic terminus of EBV episomal DNA characteristic of the original tumor.

- Blood samples not to exceed 20 ml/sample and marrow samples not to exceed 10 ml/sample will also be drawn at approximately 6 month intervals for 1 year and thereafter as part of clinically indicated evaluation to assess the presence of minimal residual disease, using pcr amplified techniques for detection of EBV DNA and, when possible, lymphoma-associated VDJ rearrangements.

Evaluations	Pre Infusion	Day 0	Day 1	Day 7	Day 14	Day 15	Wk 3	Wk 4	Wk 5***	Wk 6	Wk 7	Wk 8	Mo 3	Mo 4	Mo 5	Mo 6	Mo 7	Mo 8	Mo 9	Mo 10	Mo 11	Mo 12
Clinical Evaluation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GvHD evaluation	X	X	X	X	X	X	X	X	X	X	X	X										
EBV specific CTLp (10-20cc blood)**	X	X	X	X	X	X	X	X	X	X			X			X			X			X
Cytokine Analysis		X		X	X																	
CBC	X	X	X	X	X	X	X	X	X	X			X			X			X			X
EBV PCR (10 ccs)	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
T cell Immunophenotype short panel	X	X	X	X	X	X	X	X	X	X			X			X			X			X
Mitogens/Antigens	X								X													
CT/PET or MRI (if indicated)	X								X							X						X
Lymph Node Biopsy(if possible)	X								X													

Blood/BM samples < 10 ml/sample																	X						X
Treatment EBV specific T cells****		X		X	X																		

APPROXIMATE SCHEDULE OF EXAMINATIONS AND STUDIES*

*Tests/examination will be performed as clinically necessary. If patient is without T-cell count, T-cell proliferation and research tests will be held

** RESEARCH NON-BILLABLE (Sodium Heparin Tubes to Dr. O'Reilly's lab: Z1645)

***Patients will go on to receive tests/examinations post-week 5 once it is determined that they will not be infused with additional EBV CTLs

****If patient is given additional cycles of EBV specific T-cells, calendar will restart from Day 0. Doses are given weekly (+/- 3 days) and can be held at the discretion of the attending in the case of toxicity

11.0 TOXICITIES/SIDE EFFECTS

11.1 Patient Toxicities/Side Effects

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

Each of the following Toxicities and Side Effects is expected to be unlikely:

1) Acute Toxicities: The EBV-specific T-cells to be used for adoptive immunotherapy are activated T-cells potentially capable of generating significant quantities of cytokine such as IL-2, IFN-γ and TNF. **These cytokines may acutely induce fever and could potentially cause hypotension and other manifestations of shock, including toxicity to the lung**. However, experience with infusions of CMV-specific T-cells (55), in vitro expanded tumor infiltrating T-cells (54) and existing limited studies of adoptively transferred EBV-reactive T-cells, either as purified fractions or in donor leukocytes (34,46, 57, 58) suggest that these infusions will be well tolerated, with a low risk of systemic toxicity.

2) Marrow or Organ Allograft Rejection: Patients receiving third party donor-derived EBV-specific T-cell infusions for treatment of monoclonal EBV-lymphomas complicating the immunoablation used to prepare patients for an allogeneic hematopoietic transplant or the immunosuppression used to sustain an organ allograft may be at risk of an acute rejection episode following infusion of the T-cells. Such episodes could be precipitated if significant numbers of alloreactive T-cells are present in the T-cell inoculum. In allogeneic hematopoietic cell transplant recipients, this could lead to loss of the hematopoietic cell transplant, resulting in marrow aplasia, a potentially lethal complication. Therefore, if severe leukopenia or thrombocytopenia is observed that is potentially attributable to the infused T-cells, the patient will be treated with prednisone and/or anti-thymocyte globulin to eliminate the infused third party T-cells.

In organ transplant recipients it is also possible that the EBV-specific T-cells, upon interaction with their targets, will generate cytokines capable of stimulating alloreactive host T-cells that have been suppressed by drugs used to prevent rejection. If a rejection crisis is precipitated, it will be treated as recommended by the patient's transplant physician.

3) Graft Versus Host Disease: Acute Graft Versus Host Disease (GvHD) represents an immune assault induced by engrafted alloreactive donor T-lymphocytes, against host alloantigens

predominantly expressed on hematopoietic cells and their tissue-based progeny. It is manifested by skin rash, hepatitis, enteritis and suppression of host hematopoietic and lymphoid tissues resulting in blood count depressions or aplasia and prolonged immunodeficiency.

The development of GvHD necessitates engraftment and proliferation of donor alloreactive T-cells. Patients who have received organ allografts and even the most severely immunodeficient AIDS patients are able to reject allogeneic lymphoid cells. Despite a large documented experience of transfusions of unirradiated cells, no instance of GvHD has been recorded in AIDS patients or recipients of kidney, lung, heart, or liver grafts (59). Thus, these patients are likely not at risk for this complication

On the other hand, marrow transplant recipients and children with severe combined immunodeficiency are at risk of acute and chronic forms of GvHD if infused with unirradiated HLA-matched or HLA-mismatched T-cells capable of responding to host alloantigens. Acute GvHD affects 60% of recipients of HLA-matched marrow grafts. Up to 85% of adults transplanted with HLA-matched marrow from unrelated donors or partially matched related donors will develop moderate to severe (grade II-IV) acute GvHD despite drug prophylaxis (60). This complication is a principal contributor to mortality in 40-50% of cases. Furthermore, 50% of the survivors of acute GvHD will develop manifestations of chronic GvHD (61).

The in vitro propagation of T-cells reactive against autologous EBV-transformed cells selects for the expansion of EBV-specific T-cells and the elimination of other T-cells, including alloantigen-responsive T-cells (58). As a result, infusions of virus-specific T-cells generated over 4-6 weeks in vitro in our own and other published experience (46, 47, 49, 53) have thus far not been complicated by GvHD in HSCT transplant recipients. Nevertheless, there is a risk that small numbers of alloreactive T-cells could be transferred with the infusions of EBV-specific T-cells. To counter this risk, only HLA matched or HLA partially matched donors will be used as donors of EBV-specific T-cells and only EBV-specific T-cells tested to be free of cells reactive against allogeneic cells will be administered to patients who could be durably engrafted following a T-cell infusion.

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

5) Transfusion Reactions: It is possible that during the course of repeated infusions of EBV-specific T-cells derived from a normal donor, the host will develop an immune response against the donor cells, which could lead to an immediate allergic response, manifested by generalized urticaria of the skin, angioneurotic edema, or more serious manifestations such as bronchospasm or hypotension. Such reactions will be treated symptomatically.

6) Treatment Failure: Treatment with EBV-specific T-cells may not alter the progression of EBV lymphoma or may induce only transient regressions of disease. Furthermore, because of host immune responses to donor cells, secondary or subsequent treatment by this adoptive immunotherapeutic approach may be precluded.

7) Reproductive Risks: Patients should not become pregnant or father a baby while on this study because the drugs in this study can affect an unborn baby. Women should not breast feed while on this study. Women may need to use birth control while on the study, but should check with their study doctor regarding what type of birth control and how long to use it.

11.1 Donor Toxicity/Side Effects

Risks of blood donation are minimal and include: minor discomfort at site of blood draw, some bleeding and/or bruising, and lightheadedness.

Risks of Leukapheresis include: Lack of feeling or tingling of fingertips or around mouth, which normally last only a short time, and can, potentially, progress to cramps. This is normally treated with a calcium supplement.

Other leukapheresis side effects include:

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

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

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

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

1) Acute toxicities induced by infusions of EBV-specific T-cells will be graded according to the NCI-CTC 4.0 criteria used to grade organ toxicities induced by chemotherapeutic agents (62).

2) Allograft rejection episodes developing in organ transplant recipients will be diagnosed, assessed, and managed by the patient's transplant physician, utilizing standard criteria established for the identification and grading of rejection episodes in transplanted kidney, liver, heart or lung allografts (63-65).

3) Acute Graft vs. Host Disease will be diagnosed and graded utilizing the IBMTR consensus criteria (66) and the histopathological criteria of Slavin and Woodruff (67). Chronic graft vs. host disease will be diagnosed and graded using the staging criteria developed by Sullivan et al (68).

4) The responses of the clinically and/or radiologically evident EBV LPD or malignancies will be identified three weeks post-infusion cycle and may be performed earlier if clinically indicated. In our experience, patients with Nasopharyngeal Carcinomas do not typically show response to treatment after one cycle. Evaluation for these patients will occur after a second cycle of cells, unless clinically indicated. Outcome responses will be captured post-first cycle and post final-cycle of infusions of EBV specific T cells. Best response observed in the full course of treatment will also be recorded as a distinct entity.

– Complete response: Complete resolution of all clinical and radiologic evidence of lymphoma, confirmed by biopsy of affected tissues when indicated, lasting for at least three weeks following completion of a course of cell infusions.

– Partial response: A 50% or greater reduction in the size of all lymphomatous lesions as determined by CT or MRI based measurements of tumor volume, which is maintained for at least three weeks following completion of a course of cell infusions. A sustained PR is defined as a PR that is sustained for ≥ 6 months.

– Stable disease: Less than 50% reduction of lymphomatous lesions without progression at any site maintained for at least 3 weeks following completion of a course of cell infusions.

– Mixed response: A 50% or greater reduction in the size of one or more lymphomatous lesions as determined by CT or MRI based on measurements of tumor volume, which is maintained for at least three weeks following completion of a course of cell infusions, with no change or progression at another site. Or a decrease in viremia coupled with stable disease of lymphomatous lesions.

– No response with disease progression: Progressive enlargement of one or more lymphomatous lesions by three weeks following a course of T-cell infusions.

5) For recipients of HSCTs, and other severely immunocompromised patients in Groups 1 and 2 (Section 9.0), who failed initial treatment with chemotherapy and/or Rituxan for their EBV malignancy and are, therefore, treated preemptively with EBV-specific T-cells because of high and increasing levels of EBV DNA in the blood, without clinical or radiological evidence of tumors, a therapeutic response will be defined as the following. Outcome responses will be captured post-first cycle and post final-cycle of infusions of EBV specific T cells. Best response observed in the full course of treatment will also be recorded as a distinct entity.

A. Viral - Complete Response: Clearance of EBV without subsequent development of EBV LPD
B. Viral – Partial Response: At least a ten-fold decrease in EBV DNA levels
C. Viral - Stable Response: Persistence of circulating EBV DNA without development of EBV LPD
D. Viral - No Response with Progression: Persistence of EBV DNA with subsequent development of EBV LPD

13.0 CRITERIA FOR REMOVAL FROM STUDY

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

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

Patients may be removed from study, and not followed further, if one or more of the following events occur:

- Significant noncompliance on the part of the patient
- Refusal of the patient to continue treatment or observations

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

14.0 BIOSTATISTICS

A phase 2 trial design will be applied to determine the efficacy of Epstein-Barr Virus Immune T-lymphocyte lines for the treatment of EBV malignancies. The evaluation of treatment will be assessed separately for 1) patients with EBV-LPD after allogeneic HSCT who are severely immunocompromised or patients with severe congenital or antineoplastic drug induced immunodeficiency and 2) patients with EBV-LPD after organ allograft recipients, AIDS patients, or patients who develop other EBV-associated malignancies without pre-existing immune deficiency. For patients with EBV-LPD, the primary endpoint is complete response or a sustained partial response for 6 months as defined in section 12.

For patients in Group 1 who have an EBV-LPD, a two-stage design with a maximum of 28 patients will be accrued onto this study. The therapy is considered active if the probability of a complete or sustained partial response for 6 months (CR/SPR) in the population exceeds 0.40. In the first stage, 16 patients will be enrolled in the study. If 6 or fewer CR/SPRs are observed, then the trial will be stopped. If at least 7 responses are observed, then 12 additional patients will be accrued, for a total of 28 patients. At the conclusion of the trial, if 14 or fewer CR/SPRs are observed, we will conclude that the treatment combination is not sufficiently active. This design has power 0.90 for a population CR/SPR proportion of 0.64 using a one-sided 0.10 size test. At the conclusion of the study, we will estimate the probability of a complete response or a sustained partial response for 6 months and the 95% confidence interval for this response parameter. Kaplan-Meier estimates of overall and disease free survival over time will be computed.

Patients in Group 2 (section 9.0 and 9.2) who develop an EBV LPD as a complication of an organ allograft or AIDS and patients with EBV-associated malignancies without pre-existing immune deficiency are expected to consistently reject the third party cells. As a result, the survival of cells post each infusion is expected to be shorter. Nevertheless, an initial expansion suggests that 50-70% of these patients may achieve a durable CR or PR. Therefore, for this group, we will also adopt a two stage design, with a maximum of 28 patients to be accrued onto this study. The therapy is again considered active if the probability of a complete or sustainable partial response for 6 months (CR/SPR) exceeds 0.20. If at the conclusion of the study 9 or more of the 28 patients remain disease free for 6 months from the start of treatment, then the treatment will be considered active. This active region is based on a one-sided 0.10 size test. The study design has power 0.90 for a six-month disease free probability equal to 0.40. At the conclusion of the study, Kaplan-Meier estimates of overall and disease free survival over time will be computed.

The effects of infusing activated EBV-specific T-cells on the general immune function of the host will be assessed by recording $CD4^+$ and $CD8^+$, $CD3^+$ T-cells, NK cells and B-cells as specified in the Schedule of Examinations and Studies Table on Page 22. Nonparametric curve smoothing methods will be employed to estimate the population trajectory of these immunologic recovery factors over time. In addition, a generalized estimating equation approach will be used to model the relationship between the immunologic reconstitution factors and complete response.

It is anticipated that accrual for both groups will be completed within 3 years.

In order to reduce patient risk, the study design includes early termination in the event of excessive graft versus host disease or early treatment related mortality during the accrual period. The stopping rules for excessive failure and the corresponding power calculations are derived separately for Group 1 and Group 2 and are given in the tables below. In the event that the stopping boundary is crossed for one of the groups, the study will continue accrual in the other group.

Group 1

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
One-year TRM	2 within the first 10 patients 3 within the first 23 patients 4 within 28 patients	0.04	0.10
		0.20	0.91
Acute GvHD (grades 3-4)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92
Chronic GvHD (extensive)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92

Group 2

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Graft failure	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92
One-year TRM	2 within the first 10 patients	0.04	0.10

	3 within the first 23 patients 4 within 28 patients	0.20	0.91
Acute GvHD (grades 3-4)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92
Chronic GvHD (extensive)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92

Patient accrual for the EBV-LPD patient cohorts is almost complete. The planned accrual for each cohort was 28 patients. To date, 20 patients in group 1 have been treated with 7 patients attaining a CR/SPR and 23 patients in group 2 have been treated with 5 patients attaining a CR/SPR. In addition, the stopping boundaries for graft failure, treatment related mortality, acute GvHD, and chronic GvHD have not been exceeded. **As a result, after the planned patient accrual is complete and the stopping boundaries have not been met, we propose to accrue an additional 28 patients in each cohort with amendment 6**. These additional patients will provide a more precise estimate of the CR/SPR rate in the two patient populations. With 56 patients, the probability of a CR/SPR can be estimated to within +/- 0.13 with 95% confidence. The stopping rules below will be used to monitor the additional 28 patients for graft failure, graft versus host disease and treatment related mortality. The stopping rules for excessive failure and the corresponding power calculations are derived separately for Group 1 and Group 2 and are given in the tables below. In the event that the stopping boundary is crossed for one of the groups, the study will continue accrual in the other group.

Group 1

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
	2 within the first 10 patients	0.04	0.10

One-year TRM	3 within the first 23 patients 4 within 28 patients	0.20	0.91
Acute GvHD (grades 3-4)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92
Chronic GvHD (extensive)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92

Group 2

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Graft failure	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92
One-year TRM	2 within the first 10 patients 3 within the first 23 patients 4 within 28 patients	0.04	0.10
		0.20	0.91
Acute GvHD (grades 3-4)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92
Chronic GvHD (extensive)	2 within the first 26 patients 3 within 28 patients	0.02	0.10
		0.15	0.92

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at PPD. Registrations must be submitted via the PPR Electronic Registration System PPD. The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

N/A

16.0 DATA MANAGEMENT ISSUES

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

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

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

16.1 Quality Assurance

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

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

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at:
PPD

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: Data and Safety Monitoring Committee (DSMC) for Phase I and II clinical trials, and the Data and Safety Monitoring Board (DSMB) for Phase III clinical trials, report to the Center’s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

- **Consent process:** Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent, which must conform to MSKCC IRB guidelines.

- **Benefits:** It is not known whether this treatment will improve the overall survival of the patient.

- **Risks:** The potential risks of this therapy as described in Section 11 of this protocol may outweigh the potential benefits in an individual patient. The potential risks are related to adverse effects which could be induced by infusion of T cells derived from EBV sensitized T cell lines. Appropriate exclusion of patients are listed in the section of patient eligibility.

- **Costs:** The patient's health plan/ insurance company will need to pay for some or all of the costs of treating his/her disease in this study, including the production of EBV immune T cells. Patients will be charged for physician visits, routine laboratory and radiologic studies required for monitoring their condition.

- **Alternatives to the Planned Study:** Alternative treatment options include continued treatment with Rituxan and standard antiviral drugs used for herpes virus infections and treatment with chemotherapeutic agents used for Lymphomas not associated with EBV infection in other investigational studies. If relevant, other investigational options will also be outlined. Issues relating to best supportive care as a treatment option alone will be reviewed, given that most chemotherapy responses with standard drugs are infrequent and short lived. (see Patient Informed Consent).

- **Confidentiality:** Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential. Patient's names or any other personally identifying information will not be used in reports or publications resulting from this study. The Food and Drug Administration or other authorized agencies (e.g., qualified monitors from MSKCC or the NCI etc.), and appropriate personnel may review patient records as required.

17.1 Privacy

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

17.2 Serious Adverse Event (SAE) Reporting

17.2.1

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at PPD . The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.2 Definition of SAEs

Serious adverse event (SAE) is defined as one of the following:

• Is fatal or life-threatening
• Results in persistent or significant disability/incapacity
• Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
• Requires inpatient hospitalization or prolongation of existing hospitalization,
• Note that hospitalizations for the following reasons should not be reported as serious adverse events:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent

- Social reasons and respite care in the absence of any deterioration in the patient's general condition
- hospitalizations or prolongation of hospitalizations, due to complications of the patient's previous therapy and/or underlying malignancy

Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event. Additionally, events relating to the patient's underlying malignancy or attributable to other therapy should not be reported serious adverse events.

Attribution:

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

Expected and Unexpected Event:

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

17.2.3 Reporting Requirements

An adverse event is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient/guardian signed ICF has been obtained. Serious Adverse Events (SAEs) are any adverse events occurring while on treatment and within 30 days of the last dose that result in any of the following outcomes (Note: Events beyond 30 days will be reported at the discretion of the PI):

UNEXPECTED EVENT:

- Grades 1-2: Adverse Event Reporting NOT required.
- Grades 3-4: Possible, probable, and definite attribution to the drug and/or device will be reported.
- Grade 5: Regardless of Attribution will be reported.

EXPECTED EVENT

- Grades 1-3: Adverse Event Reporting NOT required.
- Grade 4: Possible, probable, and definite attribution to the drug and/or device will be reported.
- Grade 5: Regardless of Attribution will be reported.

SAEs will be graded according to NCI CTCAE 4.0.

18.0 INFORMED CONSENT PROCEDURES

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

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

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

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

Participants enrolled to the “historical donor” arm are, frequently, no longer under the care of MSKCC. This is because the primary patients, for whom their donations were initially intended, have completed treatment and are no longer at risk for EBV LPD. Donors who fall into this category will be mailed a copy of the consent form to review during the consent discussion, which will occur over the phone. If they agree to 3rd party use of their cells, the consent will be signed and sent back via MSKCC provided pre-paid/addressed envelope.

19.0 REFERENCES

1. Rickinson AB. EBV infection and EBV-associated tumors. In:Minson AC, Neil JC, McRae MA, eds. *Viruses and Cancer.* Cambridge: Cambridge University Press; 1994:81-100.

2. Straus SE, Cohen JI, Tosato G, Meier J. Epstein-Barr virus infections: biology, pathogenesis and management. Ann Intern Med 118:45-58, 1992.

3. Cohen JI. Epstein-Barr virus infection. N Engl J Med. 2000 Aug 17;343(7):481-92. PMID: 10944566.

4. Tosato G, Steinberg AD, Yarchoan R, Heilman CA, Pike SE, De Seau V, Blaese RM. Abnormally elevated frequency of Epstein-Barr virus-infected B cells in the blood of patients with rheumatoid arthritis. J Clin Invest. 1984 Jun;73(6):1789-95.

5. Rickinson AB, Moss DJ. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. Annu Rev Immunol. 1997; 15: 405-431.

6. Lucas KG, Small TN, Heller G, Dupont B, O'Reilly RJ. The development of cellular immunity to Epstein-Barr virus after allogeneic bone marrow transplantation. Blood. 1996 Mar 15;87(6):2594-603.

7. Lacerda JF, Ladanyi M, Louie DC, Fernandez JM, Papadopoulos EB, O'Reilly RJ. Human Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes home preferentially to and induce selective regressions of autologous EBV-induced B cell lymphoproliferations in xenografted C.B-17 scid/scid mice. J Exp Med. 1996 Mar 1;183(3):1215-28.

8. Murray RJ, Kurilla MG, Brooks JM, Thomas WA, Rowe M, Kieff E, Rickinson AB. Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies. J Exp Med. 1992 Jul 1;176(1):157-68.

9. Murray RJ, Kurilla MG, Brooks JM, et al. Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus(EBV): implications for the immune control of EBV-positive malignancies. J Exp Med 1992; 176: 157-68.

10. MacArthur GJ, Wilson AD, Birchall MA, Morgan AJ. Primary CD4+ T-cell responses provide both helper and cytotoxic functions during Epstein-Barr virus infection and transformation of fetal cord blood B cells. J Virol. 2007 May;81(9):4766-75. Epub 2007 Feb 21.

11. Tan LC, Gudgeon N, Annels NE, Hansasuta P, O'Callaghan CA, Rowland-Jones S, McMichael AJ, Rickinson AB, Callan MF. A re-evaluation of the frequency of CD8+ T cells specific for EBV in healthy virus carriers. J Immunol. 1999 Feb 1;162(3):1827-35.

12. Yang J, Lemas VM, Flinn IW, et al. Application of the ELISPOT assay to the characterization of CD8 (+) responses to Epstein-Barr virus antigens. Blood 2000; 95: 241-8.

13. O'Reilly RJ, Small TN, Papadopoulos EB, Lucas K, Lacerda J, Koulova L. Adoptive immunotherapy for EBV-associated lymphoproliferative disorders complicating marrow allografts. Springer-Verlag. Edited by G Klein, Springer Seminars in Immunopathology, 20:455-491, *1998*

14. Khan G, Coates PJ. The role of Epstein-Barr virus in the pathogenesis of Hodgkin's disease. J Pathol. 1994 Nov;174(3):141-9.

15. Burgos JS. Involvement of the Epstein-Barr virus in the nasopharyngeal carcinoma pathogenesis. Med Oncol. 2005;22 (2):113-21.

16. Suankratay C, Shuangshoti S, Mutirangura A, Prasanthai V, Lerdlum S, Shuangshoti S, Pintong J, Wilde H. Epstein-Barr virus infection-associated smooth-muscle tumors in patients with AIDS. Clin Infect Dis. 2005 May 15;40(10):1521-8. Epub 2005 Apr 12.

17. Swerdlow SH. Post-transplant lymphoproliferative disorders: A morphologic, phenotypic and genotypic spectrum of disease. Histopathol 20:373-385, 1992.

18. Hanto DW, Gajl-Peczalaska KJ, Frizzera G, et al. Epstein-Barr- virus induced polyclonal and monoclonal B-cell lymphoproliferative diseases occurring after renal transplantation. Ann Surgery 198:356-369, 1983.

19. Dharnidharka VR, Tejani AH, Ho PL, et al: Post-transplant lymphoproliferative disorder in the United States: young Caucasian males are at highest risk. AM J Transplant 2002 Nov;2(10):993-8.

20. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 323:1723-1728, 1990.

21. Patton DF, Wilkowski CW, Hanson CA, et al. Epstein-Barr virus-determined clonality in post-transplant lymphoproliferative disease. Transplantation 49:1080-1084, 1990.

22. Starzl TE, Nalesnik MA, Porter KA, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporine-steroid therapy. Lancet 1:583-587, 1984.

23. Leblond V, Dhedin N, Mamzel Bruneel MF, et al: Identification of prognostic factors in post-transplantation lymphoproliferative disorders in 61 patients with posttransplantation lymphoproliferative disorders. J Clin Oncol 19:772-778, 2001

24. Straus SE, Cohen JI, Tosato G, Meier J. Epstein-Barr virus infections: biology, pathogenesis and management. Ann Intern Med 118:45-58, 1992.

25. Kuehnle I, Huls MH, Liu Z, et al. CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hematopoietic stem-cell transplantation. Blood. 2009;95:1502-1505.

26. Papadopoulos EB, Ladanyi M, Emanuel D, Mackinnon S, Boulad F, Carabasi MH, Castro-Malaspina H, Childs BH, Gillio AP, Small TN, Young JW, Kernan NA, O'Reilly R.J. Infusions of donor leukocytes as treatment of Epstein-Barr virus associated lymphoproliferative disorders complicating allogeneic marrow transplantation. NEJM 330:1185-1191, 1994.

27. Antin J, Bierer B, Smith B, Ferrara J, Guinan EC, Sieff C, Golan DE, Macklis RM, Tarbell NJ, Lynch E, Reichert TA, Blythman H, Bouloux C, Rappeport JM, Burakoff SJ, Weinstein HJ.

Selective depletion of bone marrow T lymphocytes with anti-CD5 monoclonal antibodies: Effective prophylaxis for graft-versus-host disease in patients with hematologic malignancies. Blood 78:2139-2149, 1991.

28. Martin PJ, Shulman HM, Schubach WH, Hansen JA, Fefer A, Miller G, Thomas ED. Fatal Epstein-Barr virus-associated proliferation of donor B-cells after treatment of acute graft-versus-host disease with a murine anti-T-cell antibody. Ann Int Med 101:310-315, 1984.

29. O'Reilly RJ, Small TN, Papadopoulos EB, Lucas EB, Lasered J, Koulova L. Adoptive immunotherapy for EBV lymphoproliferative disorders complicating marrow allografts. Springer Seminars in Immunopathology, Springer Verlag. Edited by G Klein. 20:455-491, 1998.

30. Curtis RE, Travis LB, Rowlings PA, Socié G, Kingma DW, Banks PM, Jaffe ES, Sale GE, Horowitz MM, Witherspoon RP, Shriner DA, Weisdorf DJ, Kolb HJ, Sullivan KM, Sobocinski KA, Gale RP, Hoover RN, Fraumeni JF Jr, Deeg HJ. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood. 1999 Oct 1;94(7):2208-16.

31. Verschuur A, Brousse N, Raynal B, Brison O, Rohrlich P, Rahimy C, Vilmer E. Donor B cell lymphoma of the brain after allogeneic bone marrow transplantation for acute myeloid leukemia. Bone Marrow Transplant. 1994 Sep;14(3):467-70.

32. Lucas KG, Small TN, Heller G, Dupont B, O'Reilly RJ. The development of cellular immunity to Epstein-Barr virus after allogeneic bone marrow transplantation. Blood. 1996 Mar 15;87(6):2594-603.

33. Ziegler JL, Beckstead JA, Volberding PA, Abrams DI, Levine AM, Lukes RJ, Gill PS, Burkes RL, Meyer PR, Metroka CE, Mouradian J, Moore A, Riggs SA, Butler JJ, Cabanillas F, Hersh E, Newell GR, Lauberstein LJ, Knowles D, Odajnyk C, Raphel B, Koziner B, Urmacher C and Clarkson BD. Non-Hodgkin's lymphoma in 90 homosexual men. NEJM 311:565-570, 1984.

34. Roithmann S, Toledano M, Tourani JM, Raphel M, Gentilini M, Gastaut JA, Armengaud M, Morlat P, Tilly H, Dupont B, Taillan B, Theodore C, Donadio D and Andrieu JM. HIV-associated non-Hodgkin's lymphomas: Clinical characteristics and outcome. The experience of the French Registry of HIV-associated Tumors. Ann Oncol 2:289-295, 1991.

35. Gisselbrecht C, Oksenhendler E, Tirelli U, Lepage E, Gabarre J, Farcet JP, Gastaldi R, Coiffer B, Thyss A, Raphael M and Monfardini S. Human immunodeficiency virus-related lymphoma treatment with intensive combination chemotherapy. Amer J Med 95:188-196, 1993.

36. Knowles DM, Chamulak GA, Subar M, Burke JS, Dugan M, Wernz J, Slywotzky C, Pelicci G, Dalla-Favera R and Raphael B. Lymphoid neoplasia associated with the acquired immunodeficiency syndrome (AIDS). Ann Int Med 108:744-753, 1988.

37. Knowles DM, Chamulak GA, Subar M, Burke JS, Dugan M, Wernz J, Slywotzky C, Pelicci G, Dalla-Favera R and Raphael B. Lymphoid neoplasia associated with the acquired immunodeficiency syndrome (AIDS). Ann Int Med 108:744-753, 1988.

38. Baumgartner JE, Rachlin JR, Beckstead JH, Meeker TC, Levy RM, Wara WM and Rosenblum ML. Primary central nervous system lymphomas: Natural history and response to radiation therapy in 55 patients with acquired immunodeficiency syndrome. J Neurosurg 73:206-211, 1990.

39. Goldstein JD, Dickson DW, Moser FG, Hirschfeld AD, Freeman K, Llena IF, Kaplan B and Davis L. Primary central nervous system lymphoma in acquired immune deficiency syndrome. A clinical and pathological study with results of treatment with radiation. Cancer 67:2756-2765, 1991.

40. Fischer A, Blanche S, LeBidois J, Gamier JL, Niaudet P, Morinet F, LeDeist F, Fischer AM, Griscelli C, Hirn M. Anti-B-cell monoclonal antibodies in the treatment of severe B-cell lymphoproliferative syndrome following bone marrow and organ transplantation. NEJM 324:1451-1456, 1991.

41. Choquet S, Leblond V, Herbrecht R, Socié G, Stoppa AM, Vandenberghe P, Fischer A, Morschhauser F, Salles G, Feremans W, Vilmer E, Peraldi MN, Lang P, Lebranchu Y, Oksenhendler E, Garnier JL, Lamy T, Jaccard A, Ferrant A, Offner F, Hermine O, Moreau A, Fafi-Kremer S, Morand P, Chatenoud L, Berriot-Varoqueaux N, Bergougnoux L, Milpied N. Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. Blood. 2006 Apr 15;107(8):3053-7. Epub 2005 Oct 27.

42. Milpied N, Vasseur B, Parquet N, Garnier JL, Antoine C, Quartier P, Carret AS, Bouscary D, Faye A, Bourbigot B, Reguerre Y, Stoppa AM, Bourquard P, Hurault de Ligny B, Dubief F, Mathieu-Boue A, Leblond V. Humanized anti-CD20 monoclonal antibody (Rituximab) in post transplant B-lymphoproliferative disorder: a retrospective analysis on 32 patients. Ann Oncol. 2000;11 Suppl 1:113-6.

43. Moss DJ, Rickinson AB, & Pope JH. Long-term T-cell-mediated immunity to Epstein-Barr virus in man. I. Complete regression of virus-induced transformation in cultures of seropositive donor leukocytes. Int. J Cancer 22, 662-668.

44. Bonini C, Ferrari G, Verzeletti S, Servida P, Zappone E, Ruggieri L, Ponzoni M, Rossini S, Mavilio F, Traversari C, Bordignon C. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. Science. 1997 Jun 13;276(5319):1719-24.

45. Lucas KG, Burton RL, Zimmerman SE, et al. Semiquantitative Epstein-Barr virus (EBV) polymerase chain reaction for the determination of patients at risk for EBV-induced lymphoproliferative disease after stem cell transplantation. Blood. 2000; 95; 807-814.

46. Rooney CM, Smith CA, Ng CYC, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr cirus-induced lymphoma in allogeneic transplant recipients. Blood. 1998; 92: 1549-1555.

47. O'Reilly RJ, Small TN, Papadopoulos E, Lucas K, Lacerda J, Koulova L. Biology and adoptive cell therapy of Epstein-Barr virus-associated lymphoproliferative disorders in recipients of marrow allografts. Immunol Rev. 1997 Jun;157:195-216.

48. Gottschalk S, Heslop HE, Rooney CM. Adoptive immunotherapy for EBV-associated malignancies. Leuk Lymphoma 2005; 46: 1-10.

49. Comoli P, Basso S, Labirio M, et al. T cell therapy of Epstein-Barr virus and adenovirus infections after hematopoietic stem cell transplant. Blood Cells Mol Dis 2008; 40: 68-70.

50. Bollard CM, Gottschalk S, Leen AM, et al. Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. Blood 2007; 110: 2838-45.

51. Lee SP. Nasopharyngeal carcinoma and the EBV-specific T cell response: prospects for immunotherapy. Semin Cancer Biol 2002; 12:463-71.

52. Haque T, Wilkie GM, Taylor C, et al. Treatment of Epstein-Barr-virus-positive post transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. Lancet 2002; 360: 436-42.

53. Haque T, Wilkie GM, Jones MM, Higgins CD, Urquhart G, Wingate P, Burns D, McAulay K, Turner M, Bellamy C, Amlot PL, Kelly D, MacGilchrist A, Gandhi MK, Swerdlow AJ, Crawford DH. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood. 2007 Aug 15;110(4):1123-31. Epub 2007 Apr 27.

54. Wilkie GM, Taylor C, Jones MM, Burns DM, Turner M, Kilpatrick D, Amlot PL, Crawford DH, Haque T. Establishment and characterization of a bank of cytotoxic T lymphocytes for immunotherapy of epstein-barr virus-associated diseases. J Immunother. 2004 Jul-Aug;27(4):309-16.

55. Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. Science 257:238-241, 1992.

56. Rosenberg SA, Packard BS, Aebersold PM, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma: Special report. N Engl J Med 319:1676-1680, 1988.

57. Papadopoulos EB, Ladanyi M, Emanuel D, Mackinnon S, Boulad F, Carabasi MH, Castro-Malaspina H, Childs BH, Gillio AP, Small TN, Young JW, Kernan NA, O'Reilly R.J. Infusions of donor leukocytes as treatment of Epstein-Barr virus associated lymphoproliferative disorders complicating allogeneic marrow transplantation. NEJM 330:1185-1191, 1994.

58. Rooney CM, Smith CA, Ng CYC, Loftin S, Li C, Krance RA Brenner MK, Heslop HE. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 345:9-13, 1995.

59. Greenbaum BH. Transfusion-associated graft-versus-host disease: Historical perspectives, incidence, and current use of irradiated blood products. J Clin Oncol 9:1889-1902, 1991.

60. Kernan NA, Bartsch G, Ash RC, et al. Retrospective analysis of 462 unrelated marrow transplants facilitated by the National Marrow Donor Program (NMDP) for treatment of acquired and congenital disorders of the lymphohematopoietic system and congenital metabolic disorders. N Engl J Med 328:592-602, 1993.

61. McGlave P, Bartsch G, Anasetti C, Ash R, Beatty P, Gajewski J, Kernan NA. Unrelated donor marrow transplantation therapy for chronic myelogenous leukemia: Initial experience of the National Marrow Donor Program. Blood 81(2):543-550, 1993.

62. Appendix A: Grading of Toxicity. In: Robert E. Wittes (Ed): Manual of Oncologic Therapeutics. Philadelphia, PA, 1990.

63. Billingham ME, Cary NRB, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart rejection study group. J Heart Transplant 9:587-593, 1990.

64. Adams D, Newberger J. Patterns of graft rejection following liver transplantation. J Hepatol 10:113, 1990.

65. Morris PJ. The clinical and laboratory diagnosis of graft rejection. In: Brent L, Sells RA (Eds): Organ transplantation: current clinical and immunological concepts. London: Balliere Tindall, 1989.

66. Rowlings, P. A., D. Przepiorka, et al. (1997). "IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade." Br J Haematol 97(4): 855-864

67. Slavin RE, Woodruff JM. The pathology of bone marrow transplantation. Path Ann 91:291, 1974.

68. Sullivan KM, Shulman HM, Storb R, Weiden PL, Witherspoon RP, McDonald GB, Schubert MM, Atkinson K, Thomas ED. Chronic graft-versus-host disease in 52 patients: Adverse natural course and successful treatment with combination immunosuppression. Blood 57:267, 1981.

69. Comoli P, Pedrazzoli P, Maccario R, Basso S, Carminati O, Labirio M, Schiavo R, Secondino S, Frasson C, Perotti C, Moroni M, Locatelli F, Siena S. Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein-Barr virus-targeted cytotoxic T lymphocytes. Laboratorio di Ricerca Area Trapianti e Oncoematologia Pediatrica, IRCCS Policlinico S. Matteo, V.le Golgi 19, 27100 Pavia, Italy

70. Barker JN, Doubrovina E, Sauter C, Jaroscak JJ, Perales MA, Doubrovin M, Prockop SE, Koehne G, O'Reilly RJ. Successful treatment of Epstein-Barr virus (EBV)-associated post-transplant lymphoma after cord blood transplant using third-party EBV-specific cytotoxic T-lymphocytes. Blood. 2010 Sep 8. [Epub ahead of print]

71. Vaysberg M, Balatoni CE, Nepomuceno RR, Krams SM, Martinez OM. Rapamycin inhibits proliferation of Epstein-Barr virus-positive B-cell lymphomas through modulation of cell-cycle protein expression. Transplantation. 2007 Apr 27;83(8):1114-21.

72. Doubrovina E, Oflaz-Sozmen B, Kernan NA, et al. Adoptive transfer of EBV-specific T-cells for treatment of primary and Rituxan resistant EBV lymphomas following allogeneic stem cell transplants (HSCT): clinical, viral and immunologic corelates. Biol Blood Marrow Transplant 2010;16(2): S220-S21.

73. O'Reilly RJ, Hasan A, Doubrovina E, Koehne G, Prockop S. Novel strategies for adoptive therapy following HLA disparate transplants. Best Prac & Res Clin Hem. In Press, 2011

74. Gottschalk S, Ng CY, Perez M, Smith CA, Sample C, Brenner MK, Heslop HE, Rooney CM. An Epstein-Barr virus deletion mutant associated with fatal lymphoproliferative disease unresponsive to therapy with virus-specific CTLs. Blood. 2001 Feb 15;97(4):835-43.

20.0 APPENDICES

N/A