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TITLE: **An Evaluation of the Toxicity and Therapeutic Effects of Epstein-Barr Virus-Immune T-Lymphocytes Derived from a Normal HLA-Compatible or Haplotype-Matched Donor in the Treatment of EBV-Associated Lymphoproliferative Diseases or Malignancies and Patients with Detectable Circulating Levels of EBV DNA who are at High Risk for EBV-Associated Lymphoproliferative Diseases**

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For donor consents and patient assessment consents ONLY: PPD PPD , PPD , , PPD

OBJECTIVES:

1) To evaluate in a Phase I/II dose escalating trial both the toxicities and therapeutic potential of adoptive immunotherapy with EBV-specific cytotoxic T-cells derived from HLA histocompatible or at least HLA-haplotype matched related donors in the treatment of EBV-induced lymphomas or other EBV-associated malignancies in severely immunocompromised hosts and organ allograft recipients who are at high risk for this complication, and to complete a single selected dose level Phase II extension of this study to identify the probability of achieving a CR of EBV lymphoma with EBV-specific T-cell therapy in allogenic hematopoietic stem cell transplant (HSCT) recipients and immunodeficient patients.

2) To evaluate the in vivo biodistribution, expansion and duration of engraftment of successive doses of transferred EBV-reactive lymphocytes within immunocompromised histocompatible or HLA-haplotype matched hosts afflicted with EBV-associated lymphoproliferative diseases and to correlate these findings with the diseased hosts' T-cell populations, general immune status and capacity to generate allospecific anti-donor response.

3) To determine the incidence, kinetics and durability of pathological and/or clinical responses of EBV-induced lymphomas to treatment with infusions of EBV-specific T-cells derived from histocompatible EBV-seropositive related donors.

BACKGROUND:

EBV-associated lymphoproliferative disorders (EBV-LPD) constitute a frequent and often lethal hematological complication of profound cell mediated immunodeficiencies, such as are observed in children afflicted with lethal genetic disorders of immunity such as severe combined immune deficiency (SCID) (1,2), Wiskott-Aldrich Syndrome (1,2) and ataxia-telangiectasia (3), recipients of marrow and organ allografts (4-6), and patients with AIDS (7-10).

Among patients with SCID, Wiskott-Aldrich Syndrome, and ataxia telangiectasia, the incidence of EBV-induced polyclonal lymphoproliferative disorders and chemotherapy refractory monoclonal EBV lymphomas has been estimated at 3%, ranging from 15% for Wiskott-Aldrich Syndrome and 11% for ataxia telangiectasia to 1.6% for SCID (11). Treatment with B-cell specific monoclonal antibodies has induced durable remissions in a proportion of patients with Wiskott-Aldrich Syndrome developing polyclonal EBV-lymphoproliferations (12). However, no therapy has altered the progression and lethality of monoclonal EBV lymphomas. Among children afflicted with the X-linked lymphoproliferative syndrome, EBV-induced hepatitis induces mortality in up to 70%, and an additional 23% develop monoclonal EBV lymphomas of the intestine which are also invariably lethal (13).

Approximately 3% of patients with AIDS will also present with non-Hodgkin's lymphoma (NHL) at diagnosis (9). The actuarial risk for this complication among symptomatic patients with AIDS receiving AZT therapy has been estimated to be 46% for those surviving 36 months on

AZT therapy (10). Of the NHL detected in patients with AIDS, 40-66% have been found to contain EBV DNA (14-16). Strikingly, virtually all primary lymphomas of the brain occurring in AIDS patients contain EBV DNA (17-18). The EBV^+ lymphomas in AIDS cases are high grade, bearing the histological features of diffuse large cell, small non-cleaved or immunoblastic B-cell lymphomas, which express not only EBNA-1 as is seen in Africa Burkitt's lymphoma but also EBNA-2, EBNA-3 and the EBV encoded latent membrane protein (LMP-I) (15-16). Although both polyclonal and monoclonal EBV^+ lymphomas have been recorded in patients with AIDS, monoclonal disease is more commonly observed (16,19). That transformation by EBV contributes to lymphomagenesis in these patients is suggested, first by the detection of EBV DNA in over 40% of nodes from HIV infected patients with persistent generalized adenopathy and its close correlation with subsequent lymphoma development (20) and second, by the detection of only a single episomal form of EBV in the lymphomas, implying that EBV infection precedes transformation (19).

Current treatment of systemic EBV^+ lymphomas developing in AIDS patients with combination chemotherapies used to treat NHL, such as CHOP, PROMACE CYTOBOM, and m-BACOD when administered with or without acyclovir or other anti-virals, has been ineffective. While 20-72% of these patients may achieve completion remissions (7, 21-24), these remissions are short lived. Furthermore, intensive chemotherapy has been complicated by a high mortality ascribable to opportunistic infections. As a consequence, median duration of survival post-diagnosis of these lymphomas has ranged between 3-15 months (7,21-24). For AIDS patients with lymphomas of the central nervous system, which are consistently EBV^+, median survival duration is only 2-3 months (25-26).

EBV-LPD are particularly common among organ allograft recipients. Among renal allograft recipients their incidence ranges from 0.3 - 1% (4,6,27). However, in more heavily immunosuppressed liver and heart allograft recipients, the incidences are 5% and 10-13% respectively (28-30). The EBV-LPD developing in these patients are most commonly polyclonal (31). Such polyclonal disorders may regress spontaneously if treatment with immunosuppressive drugs is curtailed (28). Alternatively, treatment with interferon-, acyclovir or B-cell specific monoclonal antibodies may induce durable remissions in a proportion of cases refractory to this approach (5,32-33). However, for patients developing monoclonal EBV lymphomas, none of these approaches has been effective.

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 (34-36) or application of more intensive, T-cell targeted, cytoreductive regimens to ensure engraftment or prevent GvHD (35). 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 high-grade diffuse large cell malignant B-cell lymphomas which are oligoclonal or monoclonal (37). Their clinical course is fulminant and usually fatal. Like the EBV^+ lymphomas which emerge in the context of profound cell mediated immune deficits, these lymphomas express not only EBNA-1 but also EBNA-2, EBNA-3 and LMP-1 (8). Like the EBV lymphomas in AIDS patients, and unlike the EBV-LPD in organ graft recipients, these lymphomas usually do not respond to conventional chemotherapy, interferon or high-dose acyclovir (5,34). While these EBV-lymphomas may initially respond to B-cell specific

antibodies in up to 70% of cases, responses are sustained in only 50% of patients. In those who relapse or fail to respond to Rituximab, these lymphomas have been consistently lethal.

A striking feature of the EBV lymphomas emerging in marrow allograft recipients is that they are almost invariably of marrow donor rather than host origin (34,35), and that they occur only in intervals between completion of pre-transplant immunoablative therapy and the reconstitution of functional donor T-cell derived immunity 6-8 months post-transplant. These malignancies thus reflect the rapid emergence of EBV transformed B-cell clones from the marrow graft itself.

Our group has now treated a series of 26 patients who developed EBV-LPD following T-cell depleted marrow grafts. Four patients died of antecedent complications too early to be evaluated. Of 22 evaluable patients, 20 have achieved complete and durable remissions of disease following infusions of small doses of peripheral blood mononuclear cells derived from their EBV seropositive marrow donors (34, 58). Each patient presented with a diffuse large cell lymphoma of B-cell phenotype. The lymphomas were of donor origin in each of 11 cases evaluated. EBV DNA was detected by pcr in all cases tested. Evidence of clonality was demonstrated in 12 of 16 cases adequate for study either by clonal rearrangement of immunoglobulin genes or by size homogeneity of genomic termini of EBV episomal DNA.

The patients were treated with single infusions of peripheral blood mononuclear cells from their normal seropositive marrow donors providing doses of 2.1-10.0 x 10^5 $CD3^+$ T-cells/kg to recipients of unrelated marrow grafts and 8-10 x 10^5 $CD3^+$ T-cells/kg to recipients of HLA-matched sibling grafts. The doses administered were calculated to provide a dose of T-cells 10-fold higher than the threshold dose for acute GvHD in HLA-matched sibling recipients, but still 10-fold lower than that provided by an unmodified graft. The infusions were well tolerated. Complete pathological and/or clinical resolution of the EBV lymphomas were observed in 20/22 patients. These responses were first documented pathologically 8-16 days post-infusion. Clinical remissions were achieved within 14-30 days. Thirteen of the 20 patients have survived in sustained remission with no further treatment for 10+ to 20+ months since leukocyte infusion. The other 7 patients died of their leukemia or other transplant associated complications. Two patients died 8 and 16 days post-infusion from sepsis and interstitial pneumonia, respectively. At autopsy, there was no microscopic evidence of residual lymphoma. Ten of the patients developed GvHD (5-grade I, II or III acute GvHD; 5-extensive chronic GvHD) which responded to topical or systemic steroids not initiated until at least 4 weeks post-resolution of the lymphomas. Among these dramatic responses, one patient is particularly notable in that he presented with axillary and cervical adenopathy and rapid onset of a left hemiparesis and changes in the sensorium which progressed to a left hemiparalysis and coma. MRI scans revealed multiple lesions in the frontal cortex and diencephalon as well as one lesion in the spinal cord. These lesions also responded to the infusions and almost completely resolved by 12 months post-infusion. The patient's neurologic status rapidly improved and he became alert and communicative, with a residual moderate hemiparesis.

Among patients receiving donor leukocyte infusions for treatment or prophylaxis of EBV-associated LPD, marked increases in the number of $CD3^+$ T-cells are seen as early as 14-21 days post-infusion. T-lymphocyte responses to CD3/TCR activation by the murine anti-CD3 producing hybridoma cell line OKT3 and to stimulation with PHA also increase within 14 days after donor leukocyte infusions as indicated by proliferation. These responses contrast sharply with the negligible responses to these stimuli detected in patients not treated with PBMC. PBL

isolated from the blood of patients treated with leukocytes also generate IL-2 normally, again in contrast to untreated transplant recipients at this stage post-grafting. These results thus demonstrate a striking and often prolonged effect of donor leukocyte infusions on both T-lymphocyte numbers and T-cell function in recipients of T-cell depleted marrow grafts.

Recently, we have developed and explored two assay systems to examine the cytotoxic T-lymphocyte responses to autologous EBV transformed BLCL both in the normal marrow donor and the transplanted host: 1) a bulk culture assay to measure cell mediated cytolysis against ^{51}Cr labelled EBV transformed lines and primary lymphoma cells; and 2) a limiting dilution assay to measure the frequencies of EBV-reactive CTL precursors.

Our preliminary results suggest that in some recipients of both modified and T-cell depleted marrow grafts, reemergence of EBV BLCL reactive T-cell populations may be observed as early as 3 months post-transplant while in others, such CTLp frequencies may not develop until late after transplant. They further suggest that the infusions of donor leukocytes can not only induce marked and strikingly rapid increments in the overall number and function of T-cells in the transplanted host but can also induce a rapid expansion of EBV-specific cytolytic T-cells increasing their frequency to levels equal to or in excess of those detected in immunologically normal seropositive individuals.

For example, in one patient who had received a T-cell depleted marrow graft and had developed an EBV^+ lymphoma, EBV-specific cytotoxic cells were not detectable in bulk culture, and limiting dilution analyses suggested EBV CTLp frequencies of 1:119, 150. Strikingly, as early as two weeks post an infusion of donor leukocytes providing a dose of 10^6 CD^3 T-cells/kg body weight, and containing a total dose of 1750 EBV-specific CTL precursors, T-cell concentrations in the blood had increased to $>1000/mm^3$ and the frequency of CTLp reactive against the donor's own EBV transformed B-cell line had increased to 1/10,970. This patient's EBV^+ lymphoma resolved and is no longer detectable. Two months following the donor leukocyte infusion, the frequency of EBV-reactive CTLp had increased to 1/1583 T-cells.

The effectiveness of this adoptive immunotherapeutic approach, the rapidity of the responses observed and the small doses of putative effector cells required to induce remissions of bulky, widely disseminated lymphomatous processes suggest the possibility that adoptive transfer strategies which might permit at least transient engraftment of competent EBV-specific effector cells might also induce remissions of monoclonal EBV-associated lymphomas developing in immunocompromised patients with AIDS, organ allograft recipients and children with severe congenital disorders of immunity.

At least two strategies might induce transient engraftment and effective transfer of EBV-specific HLA restricted T-cell immunity: 1) the use of isolated EBV-specific effectors from HLA-matched seropositive normal sibling donors; or 2) the use of effectors isolated from seropositive donors who are at least haplotype matched with the patient. The absence of recorded instances of acute GvHD among transfused AIDS patients and organ allograft recipients who have not been pretreated with immunoablative agents such as total body irradiation or cyclophosphamide (39), strongly suggests that they are capable of rejecting unirradiated allogeneic T-lymphocytes even late in the disease course. Nevertheless, available data indicate that lymphocytes infused into even immunologically normal individuals may persist in the circulation for 1-7 days (40) and that transplants of marrow from HLA-matched siblings infused without pre-transplant cytoreduction can survive and be detected in the circulation for at least 14 days (41).

Furthermore, infusions of HLA-matched leukocytes to patients with AIDS have been found to induce at least transient increments in T-lymphocyte populations and T-cell function and to transfer cell mediated immune responses to tetanus antigen and keyhole limpet hemocyanin (42-44). Given the rapidity of the pathological responses observed in BMT patients treated for EBV lymphomas with small numbers of EBV CTL, we hypothesize that transiently engrafted populations of HLA-matched EBV-specific T-cells might also induce a clinically beneficial result of organ allograft recipients and patients with AIDS. This approach is not without risk, however, since a proportion of the severely immunocompromised EBV lymphoma beating hosts might be durably engrafted and could develop significant GvHD if infused with significant numbers of alloreactive T-cells..

One approach which can circumvent the risk of GvHD incurred by transferring alloreactive T-cells, incorporates the use of in vitro generated HLA-restricted virus-specific T-cell lines or clones selected on the basis of their inability to react against uninfected target cells of host or donor origin. In phase I trials, donor-derived CMV-specific T-cell clones have induced significant levels of cytotoxic T-cells in HLA-matched marrow graft recipients without inducing GvHD (45). Using a similar approach, Rooney et al (46) subsequently reported successful treatment of an EBV lymphoma developing in a recipient of an HLA non-identical marrow graft with infusions of donor-derived EBV-specific T-cells generated in vivo by culturing and expanding the donor's lymphocytes with irradiated autologous EBV-transformed B-cell lines for 4-6 weeks. Alloreactive T-cells are selected against and depleted over this period of culture. Likely for this reason, GvHD was not observed. In a subsequent study, such T-cell lines derived from normal unrelated marrow donors have been administered prophylactically to an additional 49 transplanted for leukemia. In this group, no EBV lymphomas have developed. Only 1 patient experienced a mild exacerbation of pre-existing GvHD following the infusion (57), presumably as a consequence of cytokines.

In this protocol, we initially proposed a phase I/II trial designed to explore the toxicity and potential therapeutic effects of in vitro expanded EBV-specific T-cells when used for the treatment of monoclonal and otherwise refractory EBV lymphomas emerging in two classes of immunocompromised hosts: 1) marrow graft recipients and genetically immunodeficient hosts susceptible to engraftment by these cells and 2) AIDS patients and organ allograft recipients whose immune system is still adequate to prevent durable engraftment and expansion of allogeneic HLA-matched or haploidentical T-cell populations. On 8-22-06, this protocol was amended to expand the application of EBV-specific T-cells to provide preemptive treatment of those immunocompromised hosts in patient classes 1 and 2 who have high levels of EBV DNA in circulating mononuclear cells and are, therefore, at high risk of developing an EBV lymphoma.

Progress Report for The Phase I/II Trial (Reported to IRB- 7-24-08)

As written, Protocol 95-024 called for Phase I trials of EBV-specific T-cells in two stratified patient groups.

Patients in Group I included marrow graft recipients and patients with severe congenital or anti-neoplastic drug- induced immunodeficiency who would be likely to be durably engrafted and would therefore be at risk for GVHD. These patients were each to receive a series of three

doses of 1 x 10^6 EBV-specific T-cells/ kg recipient's weight, derived from either the marrow transplant donor or, for the immune deficient patients, an HLA haplotype-matched donor. Originally, a dose escalation was planned but doses of only 1 x 10^6/kg EBV/kg recipient's weight could be consistently generated in a timely fashion for the transplant donors.

The Group I stratum of the trial accrued a total of 19 patients. One patient beyond the 18 specified was treated as an IRB approval deviation. Of the 19 patients, 18 received EBV-specific T-cells from their HLA-non-identical (N-11) or HLA matched (N-7) related or unrelated HSCT donor. One patient received T-cells from an HLA-haplotype matched relative to treat an EBV lymphoma complicating a cord blood transplant. Of the 19 HSCT recipients treated, 11 achieved complete remissions of their EBV lymphoma. None of these patients recurred with EBV lymphoma. Of the 11 patients who achieved CR, 7 currently survive. The other 4 patients died late after successful treatment of their EBV lymphomas, including two who died of complications of GVHD that antedated development of EBV-LPD, (B.D., I.C.) one who died of late graft failure (C.W.) and one who died of AML-relapse (E.M.).

One patient who achieved stable disease died of neurological complication of prior CNS metastases of EBV lymphoma (C.L.).

Of the remaining seven patients, one died of multi organ failure that antedated infusion of T-cells it was too early to evaluate for EBV lymphoma response (Z.G.). Six patients (L.B., M.V., C.S., C.V.,D.A., and M.T.) died of progressive EBV lymphoma. All but one of these patients died within 28 days of initiation of EBV-specific T-cell therapy.

In each of these patients, the infusions of T-cells were well tolerated without severe toxicities. There were no instances of new onset of graft vs. host disease. Two patients with severe GVHD at the time of T-cell infusion ultimately died of their GVHD late after achieving a complete response of their EBV lymphoma. There were also no late complications that could be ascribed to the infusion of EBV-specific T-cells. This arm was closed to further accrual because of completion of accrual of the number of patients specified in the trial for the Phase I evaluation of EBV-specific T-cells in Group I patients (n=18).

The second arm of the Phase I/II trial, which is still open, is 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. These patients, unlike the marrow transplant recipients, were not expected to and did not experience sustained engraftment of the EBV-specific T-cells.

To date, six organ allograft recipients and one patient with AIDS with host type EBV lymphomas were treated with either ex vivo expanded autologous (N=2) or haplotype matched, related (N=5) EBV specific T-cells.

As specified in the protocol, each of these patients received two courses of 3 weekly doses of EBV-specific T-cells separated by a 3 week rest period. The initial doses were 1 x 10^6

EBV-specific T-cells/kg 1 week x 3 weeks. In recipients of T-cells from related donors, transient increments in circulating EBV-specific T-cells were detected after each dose of T-cells administered, but fell to low or undetectable levels by 21 days after the third weekly dose in each course administered.

The courses of these patients have been previously detailed in reports to the IRB and FDA (7-24-08). Of the 5 patients treated with EBV-specific T-cells from related HLA-haplotype matched or compatible donors, 2 organ allograft recipients achieved sustained PRs of their EBV lymphomas and are now 2 and 5 years post treatment; 2 other allograft recipients achieved S.D., of whom one has a residual EBV+ Leiomyosarcoma that has not increased in size for more than 2 years. One patient with AIDS died of progressive disease. Two patients with a history of EBV lymphoma received ex vivo expanded autologous EBV-specific-T-cells as treatment for EBV reactivation. The EBV levels reverted to undetectable in both cases and EBV lymphoma has not recurred over 1 and 10 years of follow-up respectively.

ELIGIBILITY:

Any immunocompromised patient afflicted with a pathologically documented EBV antigen positive lymphoproliferative disease, lymphoma or other EBV-associated malignancy who has an HLA compatible, or at least 2 HLA allele matched EBV-seropositive immunocompetent normal donor willing to donate lymphocytes will be considered eligible for this treatment trial. Patients developing an EBV-LPD following an allogenic HSCT from a related or unrelated donor will be eligible to receive adoptive therapy using EBV-specific T-cells generated from blood lymphocytes donated by the HSCT donor. If the donor is EBV seronegative or not available (e.g. a cord blood transplant), EBV specific T-cells generated from a normal seropositive related or unrelated donor matched for at least 2 HLA alleles may be used.

In addition, severely immunocompromised patients, as described below, who develop levels of EBV DNA in peripheral blood mononuclear cells exceeding 500 copies/ul DNA, and are therefore at high risk for developing an EBV LPD will also be eligible to receive EBV-specific T-cells generated in vitro from the blood of a suitably HLA-matched or at least partially matched EBV seropositive immunocompetent donor, or, for recipients of unrelated marrow grafts, from the unrelated marrow donor.

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

1) Patients developing **or at risk for** EBV lymphomas or lymphoproliferative disorders following an allogeneic marrow transplant. In these cases, the marrow donor, if EBV-seropositive, will be used as the donor of EBV-specific T-cells for adoptive immunotherapy, because the EBV-LPD are almost invariably derived from that marrow donor. However, if the HSCT donor is EBV seronegative or not 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 matching for 2 HLA alleles shared by the transplant donor and

recipient. However, priority is given to T cells partially 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 **or at risk for** EBV lymphomas or lymphoproliferative disorders following an allogeneic organ transplant. In these cases, the lymphoma may be derived from either donor or recipient. If the origin of the lymphoma is known, EBV-specific T-cells will be expanded from an EBV-seropositive normal donor who is at least matched for 2 HLA alleles 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 such donors would be able to recognize and kill lymphoma cells presenting EBV antigens in the context of an appropriate HLA restricting e lement.

3) Patients with AIDS developing EBV lymphomas or lymphoproliferative diseases as a consequence of the profound acquired immunodeficiency induced by HIV. For such patients, normal, EBV-seropositive related donors who are preferentially HLA compatib le or if a compatible related donor is not available, at least HLA matched for 2 alleles will be used as the source of expanded, EBV-specific T-cells.

4) Patients who develop EBV lymphomas or lymphoproliferative diseases 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-seropositive HLA compatible related or, if a compatible donor is not available, a seropositive normal donor matched for at least 2 HLA alleles will be used, as described for AIDS patients.

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 at least 2 HLA alleles 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.

EXCLUSIONS:

The following patients will be excluded from this study:

a) Moribund patients who, by virtue of heart, kidney, liver, lung, or neurologic dysfunction not related to lymphoma, are unlikely to survive the 6-8 weeks required for in vitro generation and expansion of the EBV-specific T-cells to be used for therapy and the subsequent 3 weeks required to achieve an initial assessment of the effects of infusio ns of EBV-specific T-cells.

b) Pregnancy does not constitute a contraindication to infusions of EBV-specific T cells.

TREATMENT PLAN:

I. Pre-Treatment Evaluation of Patients with, or at high risk for EBV Lymphomas Eligible for Adoptive Immunotherapy with EBV-Specific T-Cells

Immunocompromised patients presenting with detectable levels of circulating EBV DNA, who are therefore at high risk of developing an EBV-induced lymphoma and those with pathologically confirmed EBV lymphoma will be stratified according to patient group as defined in the eligibility criteria. All patients will receive a detailed clinical evaluation. EBV DNA will be quantitated in peripheral blood mononuclear cells, using a quantitative pcr-amplified assay, previously described (59, 60), incorporating appropriate controls to define EBV DNA copy number/ul of blood. In addition, radiographic analyses, if clinically indicated, including PET/CT and/or MRI scans to define extent of disease will be obtained and any lesions quantitated as to size by three-dimensional measurements. A biopsy of affected or suspicious tissue will be obtained. 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 will usually have lymph nodes assessable for such biopsies. Alternatively, needle biopsies of parenchymal lesions may be obtained. The purpose of these biopsies is to obtain viable lymphoma 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. In addition to these studies, pathologically confirmed lymphomas will be characterized by histopathologic and immunohistochemical analysis for immunophenotype and expression of EBV-associated antigens such as EBNA-1, EBNA-2 and/or LMP-1. If sufficient cells are available, they will also be evaluated for the presence of EBV-associated RNAs, EBER-1 and EBER-2. Whenever possible, an aliquot of cells will be cryopreserved.

The patients will also have blood samples drawn to evaluate:

1) <u>General immune function</u>. 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.

2) <u>EBV-specific immunity</u>. 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.

Some patients may undergo diagnostic and/or other testing of EBV involved tumor tissue to determine if their EBV disease is likely to respond to treatment with EBV specific T cells. These patients will sign a separate pre-treatment consent. If it is determined that a patient will qualify for and might benefit from infusions of EBV CTLs, he/she will go on to sign the standard treatment consent for MSKCC IRB # 95-024 and receive the pre-infusion tests detailed above.

II. Selection and Evaluation of Prospective Donors from Whose Blood EBV-Specific T-Cell Effectors Will Be Generated

Adequate health for donation as determined by institutional (related donor) or NMDP (unrelated donor) guidelines. Normal donors will be evaluated for evidence of prior sensitization to EBV by

EBV serology. They will also be typed for HLA-A,B, C and DR. For marrow transplant recipients, the marrow transplant donor will constitute the donor of choice, since the lymphomas almost invariably are of donor origin. However, if the HSCT donor is EBV seronegative or not available (e.g. a cord blood transplant), EBV-specific T-cells generated from a seropositive donor matched for at least 2 HLA alleles shared by the transplant donor and/or recipient may be used. However, priority is given to T cells partially 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. For recipients of organ allografts, selection of a donor partially matched either to the patient or the organ graft will be based on a determination of the HLA typing of the patient's lymphoma whenever possible. If the origin of the lymphoma is not determined, T cells from a donor bearing HLA alleles shared by the patient will be employed. For patients with severe primary or acquired immune deficiency disorders who, unlike organ allograft recipients, might be at risk for durable engraftment of the EBV-specific T-cells, normal, related EBV seropositive donors who are HLA compatible with the recipient will be preferentially used or, if such a donor is not available, a seropositive related or unrelated donor bearing ≥ 2 HLA alleles shared by the patient will be considered for blood donation.

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. Donors must have Hgb value > 10 gm/dl and must be capable of undergoing a single 3-6 unit leukapheresis (preferable) or a single unit of blood for T cells (<u>for pediatric donors</u>, no more than 5 ml/kg at any one blood draw). Serologic testing for transmissible diseases will be performed as per each department's guidelines or at the discretion of the treating attending.

Prospective donors will be informed of the purposes of this study, and its requirements. The donors will be requested to provide two blood samplings:

i) <u>An initial donation of 25 ml of heparinized blood</u>-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. Because 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, it is important that this sample be obtained as early as possible for patients at risk for an EBV lymphoma or other EBV- associated malignancy. Because patients receiving hematopoietic progenitor cell transplants from unrelated or HLA disparate donors are particularly at risk for EBV lymphomas in the first 2-3 months post transplant, this sample should be obtained from the donor prior to donation of the hematopoietic progenitor cell transplant whenever possible.

ii) <u>A donation of either a single standard 2 blood volume leukapheresis collected in standard ACD anticoagulant.</u> 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.

This donation of a leukapheresis or a unit of blood will be obtained from unrelated HSCT donors at least 2 weeks after their primary stem cell donation, but as soon thereafter as possible.

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 developing in the patient for whom the donor has provided an HSCT, each donor will be informed of and may provide separate consent to one, both or neither of the following potential applications of the blood cells donated:

1) 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 and b) immune T-cells specific for a protein, called WT-1, that is differentially expressed by malignant blood cells. Such T-cells could be used, under separate protocols to treat CMV infection (IRB 12-086) and/ or to treat or prevent leukemia recurrence in the patient receiving a donor's hematopoietic progenitor cell transplant. (IRB 07-055)

   Consenting to this application will limit the number of blood donations that would be required of any donor, since the white cells contained in one leukapheresis are sufficient to grow enough T- cells to treat each of the three conditions in a transplant patient.

2) The donation of any immune T-cells generated from the donor that are not used for or required by the patient for whom they were originally intended to a bank of immune cells that 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 other patients with EBV or CMV associated malignancies/infections that express HLA alleles shared by the donor's EBV or CMV-specific T-cells.

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

For the generation of EBV-specific T-cells, a series of at least two blood samplings will be required. Initially, 25 ml of heparinized blood will be 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 establishment of an EBV transformed cell line from these cultures, the 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 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 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 will be obtained from the donor whenever possible. 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 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 days, and therefore, 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, IL-2 and IL-15 are administered at 3 day intervals.

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 (if available). After in vitro propagation for 4-6 weeks, 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 (if available) or against HLA-mismatched EBV transformed B cell lines in standard 51Cr release assays.

IV. 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 name of the 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.

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

EBV-specific T-cells can be stored cryopreserved for at least 10 years. However, the period of risk for developing an EBV lymphoma following an HSCT extends to no more than 12 months post transplant by which time EBV- specific immune reactivity has recovered. If, however, the patient requires long term immunosuppressive therapy, this risk persists.

Accordingly, if the patient for whom the EBV- specific T-cells were generated is more than 12 months post transplant has reconstituted EBV specific immunity and is not receiving immunosuppressive agents, the donor's EBV- specific T-cells may with the consent of the T-cell donor be enrolled in a bank of EBV- immune T-cell designated for use in other patients who develop EBV-associated malignancies.

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.

VI. Treatment with EBV-Specific T-Cells

Each patient consenting to participate in this Phase I/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 marrow graft recipients and patients with severe congenital or antineoplastic drug induced immunodeficiency who are likely to be durably engrafted and are therefore at risk for GvHD. Group 2 will consist of organ allograft recipients, AIDS patients, and patients who develop other EBV-associated malignancies. These patients will almost invariably reject the infused T-cells and are therefore at very low risk of GvHD.

Patients in Group I will each receive a course of three weekly infusions of EBV-specific T-cells. Infusions will be administered on days 0, 7, and 14 (+/- 3days), and may be held in the case of severe toxicity related to infusion. Each weekly dose will provide 1 $x10^6$ T-cells/Kg recipient weight from the donor's EBV-specific T-cell line. After the third dose, patients will be observed for at least 3 weeks. If after 3 weeks, only 0-2 toxicity has been observed and GVHD has not developed or exacerbated, and no significant progression of the EBV lymphoma has been detected, these patients may be treated with a second course of 3 weekly infusions of EBV-specific T-cells at the same dose level. If, on the other hand, the patient has already achieved a PR or CR of the EBV lymphoma, the second course of three weekly infusions may be electively deleted. If after another 3 weeks, only 0-2 toxicity has been observed and GVHD has not developed or exacerbated, and no significant progression of the EBV lymphoma has been detected, these patients may be treated with additional courses of weekly infusions of EBV-specific T-cells at the same dose level.

The first cadre of 3- 6 patients in Group II will initially receive intravenous infusions of 10^6 EBV-specific T-cells/kg on days 0, 7, and 14 (+/- 3 days). Doses may be held in the case of

severe toxicity related to infusion. They will then be observed for three weeks and evaluated for toxicity and regression of EBV lymphoma. If at any time in the course of these infusions grade 3-4 toxicity has been observed, no further doses of cells will be administered. If however, no serious toxicity has been observed during this 3 week observation period, patients in Group II who are likely to be engrafted only transiently with these cells, may be treated with a second course of three weekly infusions of EBV-specific T-cells at the same dose of 10^6 EBV-specific T-cells/kg/dose. If grade 0-2 toxicity has been observed and no significant progression has been observed, these patients may be treated with additional courses of weekly infusions of EBV-specific T-cells at a dose of 10^6 T-cells/kg/dose, followed by a three week period of observation. At the end of this three week period, overall response will be evaluated.

The second group of three patients in Group II will receive the same schedule of intravenous infusions of EBV-specific T-cells, but the starting dose will be increased to $2x10^6$ EBV-specific T-cells/kg on days 0, 7 and 14 (+/-3days), with secondary courses administered at the same $2x10^6$/kg, based on the same criteria of response, toxicity and GvHD as used for the first six patients. Interim and overall responses will be assessed by the identical criteria.

The third group of three patients in Group II will again receive the same schedule of intravenous infusions of EBV-specific T-cells, but the starting dose will be increased to 5×10^6 EBV-specific T-cells/kg, with secondary courses administered at the same $5x10^6$ EBV-specific T-cells/kg, based on the same criteria of response, toxicity and GvHD as used for the first six patients. Again, interim and overall responses will be assessed by the identical criteria as used for the first group of patients.

Initial published studies using 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 (45). 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 donor marrow and lymphoid progenitors. On the other hand, AIDS patients and organ graft recipients, 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 II would 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.

Please note that, for all groups, a +/- 20% variability of total cell dose is allowed. In addition, if the planned dose of EBV peptide sensitized T-cells is not achieved, but the cells generated exhibit required levels of EBV specific cytotoxic activity and meet all other release criteria, or if not enough time has elapsed to evaluate dose limiting toxicity in the current dose level, the T-cell product may be administered, on a compassionate basis, at one of the lower doses specified in the protocol.

VII. 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 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 as to severity according to the IBMTR consensus criteria (47). Patients with significant (grade II or greater) GvHD 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.

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 two weeks 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, since this agent does not affect the viability or antigen-specific cytotoxic function of pre-sensitized, expanded T-cells. Treatment with systemic steroids, anti-thymocyte globulin, and/or Imuran should be discontinued wherever possible prior to initiation of T-cell infusions. 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. If possible, treatment with systemic steroids, anti-thymocyte globulin and/or Imuran should be discontinued prior to initiation of T-cell infusions. Since polyclonal EBV-lymphoproliferative diseases may regress following cessation of immunosuppression, cessation of agents other than cyclosporine 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 anti-thymocyte globulin or imuran. In such cases, doses of these agents will be reduced as 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 3000r-4000r to inactivate lymphocytes capable of initiating lethal GvHD. Blood products are irradiated in the Blood Bank, using a cesium gamma emitter.

VIII. Post-Infusion Evaluation

The evaluations below are approximate and will be performed as clinically necessary:

Patients will be evaluated by interval medical history and physical examination daily during the T-cell infusions. Vital signs will also be monitored prior to and at 1, 2, 4, and 24 hours following initiation of each infusion of EBV-specific T-cells. Thereafter, patients will be clinically evaluated at least weekly during the three week periods of observation following each course of infusions and, subsequently, at monthly intervals until 6 months post initial infusion. Patients achieving complete remission will be evaluated at six month intervals for one year thereafter, or more frequently as clinically indicated to assess duration of response. Patients achieving partial remissions or stable disease three weeks following the second course of T-cell infusions will be closely followed until first evidence of further progression, at which time alternative therapies may be initiated. Patients exhibiting disease progression within three weeks after the second course of T-cell infusions may also be considered for alternative treatments.

Patients will also be assessed for the effects of the adoptively transferred EBV-specific T-cells or circulating levels of EBV DNA. For these studies, heparinized blood (10cc) will be drawn on days 1, 7, 14, 21, and 28 post-T-cell infusion and, monthly thereafter for four months. Isolated PBMC will be assayed for EBV DNA copy number, using a standard quantitative pcr amplified assay, (59).

In addition to physical examinations **and assessments of circulating EBV DNA,** radiological studies, if indicated, including either PET/CT and/or MRI scans, will be obtained at three weeks following each course of three weekly infusions of EBV-specific T-cells, and again at six weeks following the second course of infusions. The size of each lesion will be determined by measuring diameter (length and width) and combine with views. For patients who achieve complete remission, these radiological evaluations will be obtained, if necessary, at six month intervals for one year following the initial infusion, and thereafter as clinically indicated. Patients achieving partial remission or stable disease will be followed at four month intervals following the initial infusion to assess duration of response. Radiological studies will only be performed if necessary. Patients who progress despite two courses of T-cell infusions will be assessed until three weeks following the second infusion, and thereafter as clinically indicated until alternative therapy is initiated.

If indicated, lymph nodes or parenchymal sites of lymphoma will be biopsied 2-3 weeks after the first and/or second dose 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 lymphoma. Immunohistochemical analyses will be performed to characterize cells infiltrating the biopsied tissues. The presence of cells bearing the EBV antigens EBNA-1, EBNA-2, and LMP-1 will also be assessed. When 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 lymphoma 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 10 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.

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 immediately before and 1, and 7 days following the first and third infusion of cells in each course, at days 14 and 21 following the last infusion of each course and at 1, and 4 months following completion of additional course of cells. By correlating these results with the CTLp frequency, estimates of the capacity to replicate in an allogeneic host and the half life of infused cells can be made. Flow Cytometry 7 panel will be obtained on the same days as EBV-specific CTLp testing.

The effects of infusing activated EBV-specific T-cells on the general immune function of the host will be assessed by quantitations of $CD4^+$ and $CD8^+$, $CD3^+$ T-cells, NK cells and B-cells and evaluations of T-cell proliferative responses to mitogens and non-EBV microbial antigens prior to and one week following each three week course of cell infusions and 1, 2 and 4 months following completion of the additional course of cells. Blood samples for these tests will not exceed 25 ml/sample. Whenever feasible, patients who have not received a marrow graft will also be tested at three times for evidence of T-cells derived from the EBV-specific T-cell infusion donor.

Weekly tests/exams will be performed, whenever possible, within +/-3 days. Monthly tests/exams will be performed, whenever possible, within +/- 7 days. Patients will go on to receive monthly tests/exams once it is established that they will not be infused with additional EBV CTLs.

VII. Criteria for Diagnosing and Grading Endpoints of Interest

1) Acute toxicities induced by infusions of EBV-specific T-cells will be graded according to the NCI criteria used to grade organ toxicities induced by chemotherapeutic agents (48).

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 (49-51).

3) Acute Graft vs. Host Disease will be diagnosed and graded utilizing the IBMTR consensus criteria (47) and the histopathological criteria of Slavin and Woodruff (52). Chronic graft vs. host disease will be diagnosed and graded using the staging criteria developed by Sullivan et al (53).

4) The responses of the EBV lymphomas will be identified as complete remission, partial remission, stable disease, or no response with disease progression based on the following criteria:

– Complete remission: 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 remission: 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.

– 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.

– 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 who have high and increasing levels of EBV DNA in the blood, without clinical or radiological evidence of tumors, who are treated preemptively with EBV-Specific T cells, a therapeutic response will be defined as:

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

BIOSTATISTICS

This study is being conducted on two patient groups: (1) Marrow graft recipients and patients with severe congenital or anti-neoplastic drug induced immunodeficiency who are likely to be durably engrafted and are therefore at risk for GvHD; and (2) Organ graft recipients and AIDS patients who will almost invariably reject the infused T-cells and are therefore at very low risk of GvHD. For the patients in Group 2, this is a continuing Phase I study to determine the Maximally Tolerated Dose (MTD) of EBV-specific T-cells in the treatment of EBV induced lymphomas.

In order to find the MTD for Group 2, a dose escalation scheme is being employed with patients entering in cohorts of three, at each starting dose level (see Section IV of the Treatment Plan). Dose escalation is based on the Dose Limiting Toxicity (DLT), which is defined as a grade 3 -4 toxicity at either the initial or second course of EBV-specific T-cell infusion. The dose escalation scheme for this study is a follows:

– If none of three patients experience DLT at a given dose level, then proceed to the next starting dose level.

– If one of three patients experience DLT at a given dose level, three additional patients will be treated at that dose level. If none of these three experience DLT, the dose is further escalated.

– If two or more of the six patients experience DLT at a given dose level, the MTD is defined as the previous dose level.

In the event that only three patients are treated at the MTD, an additional three patients will be treated at that level. If among these six patients, two or more experience DLT, the MTD will be lowered and the same criterion will be applied to the previous dose level.

The probability of escalating to the next higher dose level, based on the true risk of a DLT at the current level, is given in the table below. As the table shows, escalation to the next dose level is probable if the risk of DLT is low, and the likelihood of escalation decreases as the risk of DLT increases.

True Risk of Toxicity	.10	.20	.30	.40	.50	.60
Probability of Escalation	.87	.66	.42	.23	.11	.04

A minimum of 3 patients and a maximum of 18 patients will be treated in each of the two study groups. It is expected that each study will accrue 9 patients per year and will last approximately 1-2 years.

For patients in Group I, the phase II aspect of the trial will accrue a minimum of 20 patients and a maximum of 47 patients. We observed a complete remission rate using a Simon two-stage design. We will enroll 20 patients in the first stage. If 8 or more achieves CR then we will enroll 27 more for a total of 47 patients. If there are at least 20 patients achieving CR then we will recommend this regimen for further evaluation. This design has 90 % power to distinguish between CR rates of 35 % and 55 % with a Type I error of 10 %.

RISKS:

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 (45), 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) suggest that these infusions will be well tolerated, with a low risk of systemic toxicity. However, in critically ill patients, toxicities could be more frequent and severe.

2) Organ Allograft Rejection Crises: Patients receiving EBV-specific T-cell infusions for treatment of monoclonal EBV-lymphomas complicating 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. 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 with prednisone and/or anti-thymocyte globulin 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 immunodeficie nt 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 (39). Thus, these patients are likely not at risk for this complication

Marrow transplant recipients and children with severe combined immunodeficiency, on the other hand, 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 de velop moderate to severe (grade II-IV) acute GvHD despite drug prophylaxis (55). 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 (56).

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 (46). As a result, infusions of virus-specific T-cells generated over 4-6 weeks in vitro have thus far not been complicated by GvHD in marrow transplant patients. 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 compatible or HLA haplotype 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.

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.

RANDOMIZATION

N/A

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).

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.

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.

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.

PROTECTION OF HUMAN SUBJECTS:

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.

SERIOUS ADVERSE EVENT (SAE) REPORTING

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.

DEFINITION OF SAE

An SAE is an undesirable experience that meets one of the following criteria:

- Is fatal or life-threatening
- Is disabling
- Results in hospitalization or prolongation of hospitalization
- Results in a congenital anomaly or occurrence of malignancy
- Important medical event that jeopardizes the participant AND requires medical or surgical intervention to prevent one of the outcomes above *Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.*

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

UNEXPECTED EVENT:

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

EXPECTED EVENT

- Grades 1 – 3: Adverse Event Reporting NOT required.
- Grades 4 and 5: Regardless of Attribution will be reported*.

*Reportable events are those which occur within 30 days of the last dose of treatment on protocol. Events beyond 30 days will be reported at the discretion of the PI.

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