Roswell Park Cancer Institute Treatment Protocol

Protocol Number: I 00703

Protocol Title:

Cellular Infusions in Patients with Recurrent or Persistent Hematologic Malignancies After Allogeneic Stem Cell Transplant.

NCT# NCT00534118

Principal Investigator:

Philip L. McCarthy, Jr., M.D.

AMENDMENT V.13

Table of Contents

1.0) INTRODUCTION:

1.1) An anti-leukemic effect of allogeneic lymphocytes was described nearly 50 years ago [1]. The existence of a graft-versus-leukemia effect was shown by studies in which leukemic mice treated with irradiation and allogeneic bone marrow were more likely to be cured of leukemia than mice treated with irradiation and syngeneic marrow. Numerous studies over the years have further documented the existence of this effect and delineated its mechanisms in animal models [2,3].

Data accumulated in the late 1970's and 1980's strongly suggested the existence of this effect in human BMT [4-6]. Substantially higher relapse rates were observed in recipients of syngeneic grafts as opposed to allogeneic grafts; among recipients of allografts, relapse rates were higher if GVHD did not occur or if grafts were T-Cell depleted. *Horowitz et al.,* in an analysis of 2,254 patients with AML in first remission, ALL in first remission, or CML in chronic phase for the International Bone Marrow Transplant Registry (IBMTR), gave strong support to this concept[6]. These investigators studied four groups in detail: recipients of non-T-cell depleted allografts without GVHD, recipients of non-T-cell depleted allografts with GVHD, recipients of T-cell depleted allografts, and recipients of syngeneic transplants. The relative risk of relapse in AML patients was higher in syngeneic transplant recipients than in allogeneic transplant recipients. Among allograft recipients, GVHD was associated with a significantly lower relative risk of relapse in patients with ALL, AML, and CML. CML patients who received T-cell depleted allografts had a higher risk of relapse. T-cell depletion did not result in increased relapse rates in patients with ALL or AML. Further data analysis suggested different anti-leukemia mechanisms: (1) An anti-leukemia effect of GVHD (decreased risk of relapse in non-T-cell depleted allo-graft recipients with GVHD as compared with recipients of non-T-cell depleted allo-grafts without GVHD); (2) An anti-leukemic effect of allogeneic grafts independent of GVHD (increased risk of relapse in AML patients who received syngeneic transplant compared to recipients of allografts without GVHD); (3) An anti-leukemia effect independent of GVHD that is altered by T-cell depletion (CML recipients of T-cell depleted transplants with or without GVHD had higher probabilities of relapse than recipients of non-T-cell depleted allo-grafts without GVHD). Thus these data provided strong but indirect evidence of a powerful GVL effect in humans. Additionally, 2 groups have reported higher relapse rates in patients with Non-Hodgkin's lymphoma or Hodgkin's disease receiving similar chemo/radiotherapy regimens after autologous transplant (45%) as compared to allogeneic transplant (15%) suggesting a potential anti-tumor effect associated with infusion of allogeneic marrow [7,9].

Further evidence of a human GVL effect was provided by the observation of remission after discontinuation of immunosuppression and development of GVHD in patients with post-allo-graft relapse [10-11].

Based on the apparent power of the clinical GVL effect, and the presumed mediation of the effect by donor immune cells (T-cells and/or NK cells), several groups have investigated infusion of buffy coat cells obtained from the original bone marrow donor as a means of inducing a GVL effect in patients who have relapsed after allogeneic BMT [12-26]. Results obtained from several single-institution studies and two large retrospective multi-center studies can be summarized as follows:

a. The majority of patients with CML who relapse after allogeneic transplant (approximately 65%) have a complete response to donor buffy coat infusion. In almost all complete responders evaluated by PCR, molecular remissions have been documented. Responses are more likely in patients with chronic phase or cytogenetic relapse than accelerated or blastic disease. Multivariate analyses of two large data sets have not demonstrated an additive role of alpha-interferon in the development of complete response [23,27].

b. The median time to response is 3 months, with a range of 1 to 11 months.

c. Most responses in CML have been long lasting with the longest disease-free follow-up described 3+ years.

d. Approximately 60% of patients have developed GVHD. Although fatal GVHD is uncommon, significant GVHD develops in the majority of long-term responders, and the requirement for prolonged immunosuppression with associated morbidity and mortality is a major problem.

e. Pancytopenia has been observed in approximately 20% of patients. Many patients developing pancytopenia have had minimal donor engraftment by RFLP analysis at the time of buffy coat administration. It is postulated that in these patients infused donor lymphocytes eradicate recipient leukemic hematopoiesis; in the absence of sufficient donor hematopoietic cells, hematopoiesis does not recover. However, this explanation remains hypothetical. Pancytopenia has been transient in most patients, recovering without any intervention or after a short course of colony stimulating factor. However, a second stem cell infusion may be necessary in patients who have pancytopenia lasting beyond 28 days.

f. GVHD is closely associated with disease response. Nearly all compete responders have had GVHD and the statistical correlation of acute and chronic GVHD with complete remission was highly significant in one large analysis (p value <0.00001)[27].

g. In AML, ALL and MDS response rates are lower, being observed in approximately 20% of evaluable patients.[23,27]. A large percentage of patients with these diagnoses die of progressive disease within a few weeks of DLI, suggesting that large disease bulk/rapid growth may explain in part the lower response rate in these patients. Therefore, chemotherapy has been administered before DLI is some instances in an attempt to de-bulk tumor; however, too few patients have been treated in this way to allow a definitive assessment of its

usefulness. Another potential approach to this problem is to treat certain patients with molecular or cytogenetic relapse, in whom detection of disease-specific abnormalities may predict for subsequent development of hematologic disease [28,29]; this approach has been tested in only a few patients.

h. A few complete responses have been reported in multiple myeloma [21,25,27] but very few patients have been treated. In addition, confounding variables (such as potential anti-myeloma effects of concomitant alpha-interferon and of steroids used to treat GVHD) limit interpretation of responses in myeloma. Lastly, no responses have been reported in non-Hodgkin's lymphoma or Hodgkin's Disease but very few patients have been treated.

An issue that remains unsettled about DLI is the appropriate cell dose. A single 3-hour apheresis generally yields approximately 1-2 x 10^8 mononuclear cells/kg (containing approximately 50-60% T-cells). The median cell dose in most reports has been approximately 4.0 x 10^8 mononuclear cells/kg. Responses have been reported at lower cell doses. In patients with cytogenetic relapse of CML, *Mackinnon et al* reported responses at CD3+ T-cell doses as low as 1.0 x 10^7/kg [20,26]. Other CML patients, however, seemed to require cell doses as high as 5 x 10^8 CD3+ T-cells/kg. The degree of minor histocompatibility antigen disparity probably differs among different donor-recipient pairs; thus, if the target antigen is a minor histocompatibility antigen, then the cell dose resulting in effective GVL might also be expected to differ among different pairs. Cell dose might be especially critical in DLI using mismatched family members or unrelated donors. Infusion from these donors of cell doses comparable to the doses given by HLA-matched siblings might be more likely to cause severe GVHD. Thus, additional study is needed to assess safe doses in the setting of greater HLA-disparity. The doses and criteria for relapse and therapy of CML are listed in the eligibility criteria. These are based on guidelines used by the University of Oregon and the Department of Hematology, San Martino Hospital, Genova, Italy (R Maziarz, A Bacigalupo Personal Communication). We will also be using IBMTR criteria (http://www.ibmtr.org/).

The mechanism of response to DLI is uncertain [3]. Potential mediators include T cells with αβ T cell receptors, T cells with γδ receptors, NK cells, and cytokines released as a consequence of GVH reactions. Potential targets include minor histocompatibility antigens shared between leukemic and normal host tissue, and antigens unique to leukemia cells. Data in the literature do not allow definitive conclusions regarding the potential mediators and targets of the anti-tumor activity of DLI. The close correlation of response with GVHD may suggest that GVL is mediated by alloreactive T cells directed to minor histocompatibility antigens shared between normal and leukemic tissue; however this observation does not rule out other explanations, including the mediation of specific GVL by a separate effector population. It is likely that appropriate effector cell-target cell interaction involves target cell expression of certain cell surface molecules such as MHC class I and II, costimulatory molecules, adhesion molecules, and FAS[30]. Conceivably assessment

of expression of such cell surface molecules by malignant cells may predict for response or lack of response to DLI.

Allogeneic cellular therapies such as DLI are associated with life-threatening toxicities. These usually result from the development of GVHD, which results from the donor cells recognizing recipient tissues as foreign. This GVHD reaction can result in the need for immunosuppression that can result in infectious complications that can lead to mortality. Thus, this form of therapy must be given in a standard way so that results and outcomes of this therapy can be monitored prospectively. The protocol is designed and written so that a standard approach to the treatment of patients requiring DLI and that the toxicities and outcomes from DLI can be monitored. Toxicity monitoring is mandated for these types of potentially dangerous therapies. A Standard Operating Procedure (SOP) provides a template for how to perform tasks; however it does not carry the force and rigor that is required for protocol adherence. The results of DLI are reported to a transplant registry, the International Blood and Marrow Transplant Registry (IBMTR) in Wisconsin. Patient consent is required for the reportage of clinical outcome information to the IBMTR.

2.0) OBJECTIVES:

2.1) The primary objective is to determine if the complete response rate exceeds 10%.
2.2) Estimate the CR response rate.
2.3) To assess toxicity of cellular therapy.
2.4) We will accrue approximately 50 patients over 10 years.

3.0) RECIPIENT ELIGIBILITY CRITERIA:

3.1) Age <76.

3.2) Recipients must have an expected survival of at least 8 weeks.

3.3) Patient is not a candidate for repeat allogeneic transplant

3.4) Patients not candidates for repeat allogeneic transplant, chimerism status is not necessary for determining DLI eligibility

3.5) For those patients, eligible for allogeneic BMT but for whom DLI is offered as the first option, the patient should have full donor chimerism at relapse or after therapy for relapsed disease.
3.6) Original hematopoietic progenitor stem cell donor must be available for cell donation.
3.7) Minimal or no active GVHD (acute or chronic).

3.8) The following test results must be reported within 30 days of DLI. (It is recommended that testing be done within 30 days and reported within 7 days):

Absolute exclusion:
HIV 1 + II antibody

All others will be evaluated by BMT Director:
Hepatitis, B (sAg), and C
HTLV I + II
RPR

ABO/Rh and CMV IgG/IgM status must be known

3.9) Recipient must be able to give informed consent.

3.10) Creatinine must be less than 3mg/dl.

3.10a
Criteria for HLA matching to determine DLI dosing are as follows:

A & B Antigen & DRBI Allele matching qualifies as matched.
Any A, B Antigen or DRBI Allele mismatching is mismatched.
C & DQBI are not considered in the matching Algorithm.

3.11) A patient who has relapse of underlying disease after transplant and achieves remission after chemotherapy will be considered for DLI

3.12) Recipients with refractory EBV lymphoproliferative disorders or refractory EBV infection with associated pancytopenia are eligible for this protocol. Patients must be at least 30 days post allogeneic stem cell transplant. Conventional therapy includes acyclovir and immunoglobulin treatment. DLI may be given with or without Rituximab.

3.13) Recipient must be in relapse or have persistent malignancy (see below). For each of the hematologic conditions, any of the listed markers for relapse or persistent malignancy would be sufficient for relapse or persistent disease. For example, in a patient with CML, molecular relapse or cytogenetic relapse, or relapse with chronic phase/accelerated phase would be sufficient for relapse or persistent disease. These inclusion criteria are standard criteria for "relapse and persistent malignancy".

CML

a) molecular relapse- May be treated with Imatinib (Gleevec®). Prior to DLI may go onto DLI without Imatinib therapy.

BMT was non-T-cell depleted, a negative bcr/abl by PCR has been documented post-allogeneic stem cell transplant and bcr/abl is now detectable by 2 consecutive PCR determinations greater than 30 days apart.
BMT was non-T cell depleted, and bcr/abl is detectable by PCR determination at any time after day 180 post-allogeneic stem cell transplant.
If patient received Imatinib, the patient should be monitored at least 3 months before DLI is given.

b) cytogenetic relapse, after failing 3-6 months of Gleevec®.

c) relapse with chronic-phase, accelerated-phase, or blastic-phase disease, after failing 3-6 months of Gleevec®. Patient must be in chronic or accelerated phase only. Blastic phase patients must attain a second chronic phase.

AML, ALL, or MDS (No DLI before day +60 post allogeneic transplant)

molecular relapse evidenced by:
Less than 5% blasts in the bone marrow
Patient's leukemia-specific molecular abnormality is detectable by PCR analysis

cytogenetic relapse evidenced by:
Less than 5% blasts in the bone marrow
Patient's leukemia-specific chromosome abnormality detectable by standard cytogenetics at any time greater than day 60 post-transplant.

hematologic relapse evidenced by >20% blasts in bone marrow, or soft tissue recurrence:
must be treated with chemotherapy before DLI.

Multiple Myeloma (No DLI before day +60 post allogeneic transplant)

a) relapse or recurrence of M-protein after previous post-transplant documentation of disappearance of M protein by immunofixation after Thalidomide or other salvage treatment failure.
b) residual or progressive disease
c) Rising M-protein level at any time post- transplant at a 3-month interval.
Original M-protein detectable at 6 months postransplant
Residual (>5%) plasma cells in bone marrow.
d) IPEP is required to show that M-component is the same on day +60 as pre-transplant

Non-Hodgkin's Lymphoma or Hodgkin's Disease (No DLI before day +60 after Allo SCT)

a) relapse or progression of disease must be evidenced within 3 months prior to a donor lymphocyte infusion by physical exam, radiographic studies, or molecular studies. If possible, tumor should be re-biopsied to determine histology. Peripheral blood must be assayed for the EBV genome by means of EBV DNA testing by PCR, done within 30 days of DLI, if EBV lymphoma is suspected.

Epstein-Barr Virus Infection

a) viremia associated with pancytopenia
Persistent or refractory pancytopenia accompanied by EBV genome detected by PCR in the peripheral blood. Refractory pancytopenia is defined as poorly responsive to growth factors and/or transfusions.

Epstein-Barr Virus Lymphoproliferative Disorder

a) clonal lymphadenopathy that is refractory to standard therapy with acyclovir and immunoglobulin. DLI may be given with Rituximab.

4.0) EXCLUSION CRITERIA:

4.1) Pregnant or lactating women.

4.2) KPS less than 60%

4.3) CNS recurrence that is not cleared by standard chemotherapy. CNS remission status must be maintained for two weeks

4.4) Life expectancy of less than 8 weeks

4.5) Patients who fail to engraft following allogeneic SCT are not eligible for DLI.

5.0) DONOR ELIGIBILITY CRITERIA

5.1) Original stem cell donor.

5.2) Must be in good general health by history and physical examination and fulfill donor criteria as per FACT standards. We will prefer to use a RPCI clinician for donor evaluation and clearance. The criteria for donation are listed in the BMT standard operating procedures.
(http://internal.roswellpark.org/document_426_317.html)

5.3) The following tests must be collected and reported within 7 days of each DLI collection:
Absolute Exclusion:
HIV 1 + II antibody

All others will be evaluated by BMT Director:
Hepatitis B (sAg), and C
HTLV I + II
RPR
CMV IgG
IgM

ABO/Rhstatus must be known.

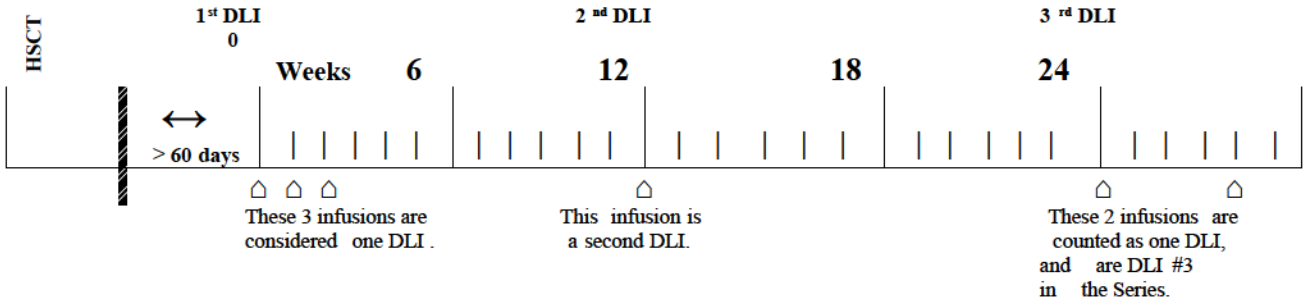
All stem cell products will be collected per current FDA Guidances. Current FDA Guidances may be referenced at the following website:

http://www.fda.gov.

5.4) Must have CBC within normal limits.
5.5) Must be able to give informed consent.
5.6) Must have negative serum pregnancy test for female donors of childbearing age.
5.7) Syngeneic donors are not eligible.
5.8) Must have good peripheral venous access, or be willing to have central venous line placement for cell collection.

6.0 PROCEDURES:

6.1) **DLI Calculation Timeline:** Some patients have cellular infusions on more than one day. A single DLI is defined as all infusions given within a 6 week period starting from the date of the first DLI > 60 days following a HSCT or 6 weeks post prior DLI. Separate DLIs are given after this 6-week period. For example:



In this example, 6 Infusions = 3 DLI accruals = 3 consents.

Infusions given ≤ 6 weeks apart are included as one DLI (one consent).
Infusions > 6 weeks apart will require reconsent and re-enrollment on study.

6.2) Protocol Date Definitions:

On-Study Date	Start Treatment Date	Stop Treatment Date	Off-Study Date
Date of first DLI infusion	Date of first DLI infusion	For multiple infusions given <6 weeks apart, date of the last infusion in the series	Date of disease restaging (~50-100 days after the last DLI infusion) or date of death due to any cause or date of onset of grade 3-4 acute GVHD, whichever comes first

6.3) GvHD Prophylaxis: Recipients will stop cyclosporine or tacrolimus. Steroids will be tapered off over 1-4 weeks as clinically indicated. A period of 2-4 weeks after discontinuation of all immunosuppression will be observed before cellular therapy is given. If GVHD flares during this period to ≥grade II acute GVHD, grade I acute GVHD requiring systemic treatment, or any extensive chronic GVHD, the patient should not receive cellular therapy for at least one month to evaluate clinical response to new GVHD.

6.4 Cellular Therapy: Cell doses for each infusion are disease-specific. Please check disease categories when determining cell dose and method of collection (see section 6.3).

6.4.1Phlebotomy will be used for patients receiving low cell doses (1 to 5 $x10^6$ $CD3^+$ cells/kg) Phlebotomy is performed by standard procedures with collection into sterile syringe or donor collection blood bags.

The first collection will be approximately 5-20 ml based on peripheral blood CD3 determination.

The second collection will be approximately 50-200 ml based on peripheral blood CD3 determination.

6.4.2 Leukapheresis procedure will be used for higher cell doses (1 $x10^7$ to 1 $x10^8$ $CD3^+$ cells/kg

Donor will sign leukapheresis consent

Peripheral venous access is preferred, but an indwelling pheresis catheter may be indicated

As many collections as needed will be done to achieve target dose.
In cases where low cell doses are stipulated by protocol, but it is anticipated subsequent doses may be required or the donor may not be available, a higher dose may be collected and cryopreserved for potential later use, at the physician's discretion.

If the original donor was from the NMDP, the NMDP cellular therapy protocol will be used for collection, and the product will be procured and given as per RPCI standards.

6.4.3 **Cells are not irradiated**. If donor and recipient are ABO incompatible, RBC packed volume infused should not exceed ≥3 ml/kg.

6.4.4 Cell Infusion

a. Flow cytometry will be done on the product to calculate the $CD3^+$ (or, T-cell) dose.
b. The cell dose will be reviewed with the Transplant Director or designee prior to infusion of cells.
c. Cells will be infused according to standard operating procedure, using standard blood filter tubing.
d. If necessary, cells can be held overnight at room temperature before infusion, kept in autologous plasma as per Processing Laboratory standards.

6.4.5 Recipient will not receive post-cellular therapy immunosuppression for GVHD prophylaxis.

6.4.6 After the first or subsequent infusion, subsequent DLI will not be given if the recipient is in remission without detectable disease and has had clinical or pathologic evidence of GVHD within 2 months of the infusion.

6.5) **Disease specific parameters for determining cell dose and content, pre-DLI chemo, and timing of infusions**:

6.5.1CML

a. pre-DLI chemo: None required. Patient may be given Gleevec or interferon to induce remission.

b. Cell dose: **HLA-matched sib or matched unrelated donor (See 3.10a)**

First infusion: $1X10^6$ CD3+ cells/kg
Second infusion: One month later, if no response, $5x10^6$ $CD3^+$ cells/kg
Third infusion: One month later, if no response (two months from first DLI), $1x10^7$ $CD3^+$ cells/kg
Fourth infusion: One month later, if no response (three months from first DLI), $5x10^7$ $CD3^+$ cells/kg

c. Cell Dose: **HLA-mismatched sib or mismatched unrelated** (See 3.10a)

First infusion: $5x10^5$ CD3+ cells/kg

Second infusion: One month later, if no response, $1x10^6$ $CD3^+$ cells/kg
Third infusion: One month later, (two months from first DLI), if no response, $5x10^6$ $CD3^+$ cells/kg
Fourth infusion: One month later, (3 months from first DLI) if no response, $1x10^7$ $CD3^+$ cells/kg.

6.5.2AML, ALL or MDS

a. Pre-DLI chemo: Recipients with molecular or cytogenetic relapse will **not** receive pre-DLI chemotherapy. Patients who are in remission from salvage chemotherapy may receive DLI. Transplant candidates should be considered for a second transplant if incomplete donor chimerism present. If not a transplant candidate, chimerism status should be know but degree of chimerism is not an eligibility criterion.

b. Recipients with hematologic relapse will receive pre-DLI chemotherapy or radiation therapy for localized recurrence. The attending physician will determine chemotherapy. Optimally, DLI will be planned for reinfusion 10-18 days after the chemotherapy has begun, i.e., when the recipient has nadired.

c. Recipients and donors must meet all eligibility criteria for DLI prior to the recipient receiving chemotherapy.

d. Recipients may receive G-CSF post chemo.

e. Cell dose: **HLA-matched sib or matched unrelated (See 3.10a)**

First infusion: $1x10^7$ $CD3^+$ cells/kg
Second infusion: One month later, if no response $5x10^7$ $CD3^+$ cells/kg
Third infusion: One month later, if no response (two months from first DLI), $1x10^8$ $CD3^+$ cells/kg
Fourth infusion: One month later, (3 months from first DLI) if no response, 1 $x10^8$ $CD3^+$ cells/kg.

f. Cell Dose: **HLA-mismatched family or mismatched unrelated (See 3.10a)**

First infusion: $1X10^6$ CD3+ cells/kg
Second infusion: One month later, if no response, $5X10^6$ $CD3^+$ cells/kg
Third infusion: One month later, (two months from first DLI), if no response, $1x10^7$ $CD3^+$ cells/kg

Fourth infusion: One month later, (3 months from first DLI) if no response, 5 x10^7 $CD3^+$ cells/kg

g. Infusion day for recipients receiving pre-DLI chemo:

Day+10 to 18 after beginning of chemotherapy (consider t1/2 of chemotherapy agents to ensure chemotherapy not in system at time of DLI). The first day of pre-DLI chemotherapy is counted as day +1.

6.5.3NHL or HD

a. Recipients and donors must meet all eligibility criteria for DLI prior to the recipient receiving chemotherapy or radiation for localized recurrence.
b. Cell dose: **HLA-matched sib or matched unrelated (See 3.10a)**

First infusion: 1x10^7 $CD3^+$ cells/kg
Second infusion: One month later, if no response 5x10^7 $CD3^+$ cells/kg
Third infusion: One month later, if no response (two months from first DLI), 1x10^8 $CD3^+$ cells/kg
Fourth infusion: One month later, (3 months from first DLI) if no response, 5 x10^8 $CD3^+$ cells/kg.

c. Cell Dose: **HLA-mismatched family or mismatched unrelated (See 3.10a)**

First infusion: 1x10^6 $CD3^+$ cells/kg
Second infusion: One month later, if no response, 5x10^6 $CD3^+$ cells/kg
Third infusion: One month later, (two months from first DLI),if no response, 1x10^7 $CD3^+$ cells/kg
Fourth infusion: One month later, (3 months from first DLI) if no response, 5 x10^7 $CD3^+$ cells/kg.

d. Infusion day:
Day +10 to 18 after beginning of chemotherapy (consider t1/2 of chemotherapy agents to ensure chemotherapy not in system at time of DLI). The first day of pre-DLI chemotherapy is counted as day +1.

6.5.4Multiple Myeloma

a. Pre-DLI chemo: None if disease controlled. Otherwise, may be given salvage chemotherapy and cells infused on day + 10 to 18 after the beginning of chemotherapy.

b. Cell dose: **HLA-matched sib or matched unrelated (See 3.10a)**

First infusion: 1x10^7 $CD3^+$ cells/kg
Second infusion: One month later, if no response 5x10^7 $CD3^+$ cells/kg

Third infusion: One month later, if no response (two months from first DLI), $1x10^8$ $CD3^+$ cells/kg
Fourth infusion: One month later, (3 months from first DLI) if no response, $5 x10^8$ $CD3^+$ cells/kg.

c. Cell Dose: **HLA-mismatched family or mismatched** unrelated (See 3.10a)

First infusion: $1x10^6$ $CD3^+$ cells/kg
Second infusion: One month later, if no response, $5x10^6$ $CD3^+$ cells/kg
Third infusion: One month later, (two months from first DLI), if no response, $1X10^7$ CD3+ cells/kg
Fourth infusion: One month later, (3 months from first DLI) if no response, $5 x10^7$ $CD3^+$ cells/kg.

7.0) DISEASE RESPONSE TO DLI: The response to therapy will be based on the response of the underlying disease and not on the basis of the development of GVHD. Disease response for all diseases is defined by the International Bone Marrow Transplant Registry (IBMTR) standards as follows:

7.1) CML:
Hematologic Remission . is described as control of the WBC count.
Cytogenetic Remission, is described when the 9;22 translocation becomes undetectable by FISH.
Molecular Remission, is described when the 9;22chromosome becomes undetectable by PCR.

7.2) AML, ALL, MDS:
Complete Remission requires that the following be maintained for at least 4 weeks.:
The peripheral blood neutrophil count should be >1.5 x 10^9/L, and platelets >100 x 10^9/L.
Bone Marrow Cellularity should be >20% and should contain less than 5% blast cells.
Extramedullary leukemia, such as CNS or soft tissue involvement, may not be present.

7.3) NHL or HD:

Complete Remission- Complete disappearance of all known disease.

Complete Remission undetermined- as above with the exception of persistent scan abnormalities of unknown significance.

7.4) Multiple myeloma:

CR= Complete Response requires **all** of the following:

Absence of the original monoclonal paraprotein in serum and urine by routine electropheresis and by immunofixation maintained for a minimum of 6 weeks. The presence of a new monoclonal band consistent with oligoclonal immune reconstitution does not exclude CR.

< 5% plasma cells in bone marrow aspirate and also on trephine bone biopsy, if biopsy is performed. If absence of monoclonal protein is sustained for 6 weeks it is not necessary to repeat the bone marrow unless the patient has had non-secretory myeloma.

No increase in size or number of lytic bone lesions on radiological investigations, (development of a compression fracture does not exclude response).

Disappearance of soft tissue plasmacytomas

For plasma cell leukemia, absence of plasma cells in blood.

(Patients in whom some, but not all, the criteria for CR are fulfilled are classified as PR, providing the remaining criteria satisfy the requirements for PR. This includes patients whom routine electrophoresis is negative but in whom immunofixation has not been performed.)

8.0) TREATMENT RESPONSE:

Patients are monitored at approximately 2week intervals as clinically indicated for:

evidence of GVHD
Disease response , including tumor measurements where indicated
toxicities
infection
second malignancies
survival

9.0) TREATMENT OF GVHD

A biopsy of the affected organ should be obtained before initiation of therapy especially for cutaneous disease. In the event that a liver or GI tract biopsy cannot be safely obtained or obtained with 48 hours, therapy may start for higher stage and grade GVHD as defined below.
For stages and grades of GVHD, topical steroids or tacrolimus may be used for initial treatment without delay.

9.1) AGVHD Gr I

Monitor without systemic therapy

9.2 AGVHD Gr II

Monitor for 1 week after onset of symptoms.
If Gr II persists for 1 week or worsens, treat with tacrolimus or cyclosporine to maintain a whole blood level of 5 to 10 ng per ml for tacrolimus and 200 to 350 ng per ml for cyclosporine.

If Gr II persists for 1 week or worsens, add intravenous methylprednisolone at 1 to 2 mg per kg or the equivalent of oral methylprednisolone, prednisone or other glucocorticoid.

9.3 AGVHD gr III and IV

Treat with methylprednisolone 2 mg/kg/d with tacrolimus or cyclosporine to maintain a whole blood level of 5 to 20 ng per ml for tacrolimus and 200 to 450 ng per ml for cyclosporine.

AGVHD not responding to initial therapy may be treated as per institutional SOP.

9.4 CGVHD-limited or extensive without GI tract or liver involvement

Treat with tacrolimus or cyclosporine to maintain a whole blood level of 5 to 10 ng per ml for tacrolimus and 200 to 350 ng per ml for cyclosporine.

If Gr II persists for 2-4 week or worsens, add intravenous methylprednisolone at 1 to 2 mg per kg or the equivalent of oral methylprednisolone, prednisone or other glucocorticoid.

9.5 CGVHD-extensive- (with GI tract or Liver involvement)

Treat with tacrolimus or cyclosporine to maintain a whole blood level of 5 to 10 ng per ml for tacrolimus and 200 to 350 ng per ml for cyclosporine and intravenous methylprednisolone at 1 to 2 mg per kg or the equivalent of oral methylprednisolone, prednisone or other glucocorticoid.

CGVHD not responding to initial therapy may be treated as per institutional SOP.

10.0) REMOVAL OF PATIENTS FROM PROTOCOL AND ADVERSE EVENT REPORTING (AER):

10.1) Removal from protocol:

If the patient experiences uncontrolled Graft Versus Host Disease, CTC Grade 4 toxicity and/or the patient no longer wishes to continue protocol therapy, the patient will be removed from the protocol. In this event the reason for withdrawal will be documented and the patient will be followed for survival. Any patient with rapid disease progression will not receive subsequent DLI.

10.2) Toxicity Reporting:

10.2.1) Expected toxicity: (recorded in protocol consent form or there is no literature).
Within 10 days of occurrence, written reports should be submitted to the IRB and the protocol chairman in the following circumstances (Bearman toxicity).

- Any fatal toxicity.
- Any non-hematologic grade 3 toxicity
- For AML, ALL, MDS recipients: slow count recovery post chemotherapy will be documented.
- Acute GVHD, Grade 3 - 4

10.2.2) Unexpected toxicity: All instances of Bearman grade 3-4 toxicity will be reported to the IRB and the protocol chairman immediately by telephone and followed by a written report.

11.0) STATISTICAL CONSIDERATIONS:

Patients who have multiple DLIs with infusions <6 weeks apart will count as one accrual, with one assessment of toxicity and response. Patients who have multiple DLIs with infusions >=6 weeks apart will count as multiple accruals, with each infusion having an assessment of toxicity and response. The response to each DLI is independent if the patient had an intervening event (disease progression or relapse) that indicated further treatment.

Let p= the proportion of patients from the heterogeneous population (AML, ALL, CML, NHL, MM patients) who achieve a complete response, CR. If it can be determined that p>0.1, then continued use of the proposed therapy will be justified. The binomial distribution will be used to test H_o: p = 0.1 versus H_1: p>0.1. If the probability of erroneously concluding H_1 is set to be 0.05 and the probability is at least 0.80 of correctly concluding H_1 when p =0.25, then a sample of n =50 patients is required. That is, if the significance level of the test is fixed to be α =0.05 and if the power of the test at 0.25 is required to be at least 0.80, then 50 patients must be accrued. Let S = the number patients

out of 50 that have a CR. If $S \geq 13$, then H_1 will be concluded. The actual significance level and power of this test are 0.05 and 0.815 respectively.

All 50 patients will be accrued even if the determination that $S \geq 4$ is made prior to the accrual of the 50th patient to increase the precision of the estimator of p. Past accruals at RPCI suggest that there will be 6 to 10 DLIs per year available to accrue to this study. Based on these data, this study will accrue patients for 6 to 10 years.

12.0) STUDY ENDPOINTS

12.1) The primary endpoint for the study is evaluating response rate, percent of complete responses, and duration of response.

12.2) The secondary endpoint is evaluation of the toxicity of cellular therapy.

13.0) DATA AND SAFETY MONITORING PLAN:

The Principal Investigator (PI) will be responsible for continuous monitoring of the safety of the study. This monitoring is accomplished by the following:

Patient Outcomes Rounds are held weekly on the transplant unit, at which time all BMT (patients receiving cellular therapy are followed as BMT patients) patient care is reviewed, including:

- medications (chemotherapy for conditioning regimens; prophylactic, empiric and therapeutic antimicrobials; graft-versus-host disease prophylactic and therapeutic medications; and possible drug interactions).
- adverse events and/or adverse reactions to any medication, procedure, or other treatment; reports are filed according to RPCI policy and procedure.
- regimen-related toxicity, based on Bearman toxicity grading, and/or Common Toxicity Criteria (CTC) if the toxicity does not correlate with a Bearman grade.
- indications for additional testing or therapies such as biopsies, scans or x-rays.
- a properly signed and dated transplant consent.
- compliance issues that could compromise patient safety.
- other aspects of safety monitoring as prescribed by the BMT Standards of Care and common clinical practice. Patients who have been discharged from the hospital are monitored in the BMT Clinic until all transplant-related issues are resolved and they are returned to the care of their referring physicians.
- Monitoring will be done according to BMT standard operating procedures: http://internal.roswellpark.org/document_426_317.html/

The BMT Quality Assurance plan requires quarterly reporting to the BMT Quality Assurance Committee, which in turn reports to the hospital Quality Assurance Committee. Indicators for BMT patient safety monitoring include:

- Patient complaints
- Adverse events and serious adverse events
- Bearman and CTC toxicity grades 3 and 4

- Variances in the delivery of standard care
- Readmissions prior to day +100 post transplant
- Deaths occurring prior to day +100 post transplant
- Engraftment

Followup on all transplant patients is continued even after they have returned to the care of their referring physicians. A Long Term Transplant Clinic has been established, which provides care for allogeneic patients with chronic complications, as well as assessments to identify dental, bone, and psychosocial complications.

All outcomes are reported to the International Bone Marrow Transplant Registry (IBMTR) the Autologous Bone Marrow Transplant Registry (ABMTR), and the National Marrow Donor Program (NMDP). Registry reports are reviewed internally prior to submission to the respective registry. These data are also entered into the RPCI BMT Database, from which patient outcomes are assessed and reviewed on a regular basis. Regimen-related toxicities reported in this fashion have resulted in a number of changes to transplant protocols since 1997, thus decreasing toxicity and improving outcomes in a number of patient groups.

Registry reports also establish the efficacy of treatment as measured by overall best response to transplant at day +100 and on subsequent annual reports. The patients' medical records serve as original source documents for all reporting. Audits are conducted every two to three years by the IBMTR and ABMTR, and the NMDP.

References:

1. Barnes D, Louti J, Neal F. Treatment of murine leukemia with x-rays and homologous bone marrow. Br Med J 2:626-630, 1956.

2. Truitt RL, LeFever AV, Shih CC-Y, Jeske JM, Martin TM. Graft-vs-leukemia effect. In: Burakoff SJ, Deeg HJ, Ferrara J, Atkinson K, (eds): Graft-vs-Host Disease. Immunology, Pathophysiology, and Treatment. New York, NY, Marcel Dekker, Inc., p 177-204, 1990.

3. Truitt R, Johnson B. Principles of graft-vs.-leukemia reactivity. Biology of Bone and Marrow Transplant 1:61-68, 1995.

4. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED, The Seattle Marrow Transplant Team: Antileukemic effect of chronic graft-versus-host disease. Contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 304:1529-1533, 1981.

5. Gale RP, Champlin RE. How does bone-marrow transplantation cure leukemia? Lancet 2:28-30, 1984.

6. Horowitz MM, Gale RP, Sondel PM et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood 75:555-562, 1990.

7. Jones RJ, Ambinder RF, Piantadosi S, Santos GW: Evidence of a graft-versus-lymphoma effect associated with allogeneic bone marrow transplantation. Blood 1991, 77(3):649-653.

8. Ratanatharathorn V, Uberti J, Karanes et al: Prospective comparative trial of autologous versus allogeneic bone marrow transplantation in patients with non-Hodgkin's lymphoma. Blood 84(4):1050-1055, 1994.

9. Petersen FB, Applebaum FR, Bigelow CL et al. High-dose cytosine arabinoside, total body irradiation and marrow transplantation for advanced malignant lymphoma. Bone Marrow Transplant 1989, 4:483-488.

10. Higano CS, Brixey M, Bryant EM et al. Durable complete remission of acute nonlymphocytic leukemia associated with discontinuation of immunosuppression following relapse after allogeneic bone marrow transplantation. A case report of a probable graft-versus-leukemia effect. Transplantation 1990, 50:175-177.

11. Collins RH, Rogers ZR, Bennett M, Kumar V, Nikein A, Fay JW. Hematologic relapse of chronic myelogenous leukemia following allogeneic bone marrow transplantation: apparent graft-versus-leukemia effect following abrupt discontinuation of immunosuppression. Bone Marrow Transplant 1992, 10: 391-395.

12. Kolb HJ, Mittermuller J, Clemm C et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood 76:2462-2465, 1990.

13. Bar BMAM, Schattenberg A, Mesnick EJBM et al. Donor leukocyte infusions for chronic myeloid leukemia after allogeneic bone marrow transplantation. J Clin Oncol 11:513-519, 1993.

14. Drobyski WR, Keever CA, Roth MS et al. Salvage immunotherapy using door leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: Efficacy and toxicity of a defined T-cell dose. Blood 82:2310-2318, 1993.

15. Hertenstein B, Wiesneth M, Novotny J, Bunjes D, Stefanic M, Heinze B, Hubner G, Heimpel H, Arnold R. Interferon- and donor buffy coat transfusions for treatment of relapsed chronic myeloid leukemia after allogeneic bone marrow transplantation. Transplantation 56(5):1114-1118, 1993.

16. Szer J, Grigg AP, Phillips GL, Sheridan WP. Donor leucocyte infusions after chemotherapy for patients relapsing with acute leukemia following allogeneic BMT. Bone Marrow Transplant 11:109-111, 1993.

17. Helg C, Roux E, Beris P et al. Adoptive immunotherapy for recurrent CML after BMT. Bone Marrow Transplant 12:125-129, 1993.

18. Porter DL, Roth MS, McGarigle C, Ferrara JLM, Antin JH. Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. N Engl J Med 330:100-106, 1994.

19. Van Rhee F, Lin F, Cullis JO et al. Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: the case for giving donor leukocyte transfusions before the onset of hematologic relapse. Blood 83; 3377-3383, 1994.

20. Mackinnon S, Papadopoulos E, Carabasi MH, Reich L, Collins NH, Boulad F, Castro-Malaspina HC, Childs BH, Gillio AP, Kernan NA, Small TN, Young JW, O'Reilly RJ. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood 86:1261-1268, 1995.

21. Collins R, Pi□eiro L, Nemunaitis J, Jain V, Waxman D, Miller W, Fay J. Donor buffy coat infusion in the treatment of persistent or recurrent malignancy after allogeneic bone marrow transplantation. Transfusion 35:891-898, 1995.

22. Giralt S, Hester J, Huh Y, Hirsch-Ginsberg C, Rond□n G, Seong D, Lee M, Gajewski J, Besien KV, Khouri I, Mehra R, Przepiorka D, K□rbling M, Talpaz M, Kantarjian H, Fischer H, Deisseroth A, Champlin R. CD8-depleted donor lymphocyte infusion as

treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation. Blood 86:4337-4343, 1995.

23. Kolb HJ, Schattenberg A, Goldman JM et al. Graft-versus-Leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 86(5):2041-2050, 1995.

24. Slavin S, Naparstek E, Nagler A, et al. Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse after allogeneic bone marrow transplantation. Blood 87:2195-2204, 1996.

25. Tricot G, Vesole DH, Jagannath S, Hilton J, Munshi N, Barlogie B. Graft-versus-myeloma effect: proof of principal. Blood 87(3):1196-1198, 1996.

26. Mackinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, Boulad F, Castro-Malaspin H. Childs B,Gillio A, Hernan NA, Small T, Young J, O'Reilly RJ. Donor leukocyte infusions for molecular or cytogenetic relapse of CML following allogeneic BMT: graft-versus-leukemia responses with minimal toxicity. Blood 86(10):566a, 1995.

27. Collins R, Shpilberg O, Drobyski W et al. Donor leukocyte infusion in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. (submitted for publication)

28. Mackinnon S, Heller G. The polymerase chain reaction is highly predictive of relapse following T-cell depleted allogeneic BMT for CML and identifies patients who may require immunotherapy with donor leukocytes. Blood 86(10):292a, 1995.

29. Radich JP, Gehly G, Gooley T et al. Polymerase chain reaction detection of the brc-abl fusion transcript after allogeneic marrow transplantation for chronic myeloid leukemia: Results and implication in 346 patients. Blood 85(9):2632-2638, 1995.

30. Guinan EC, Gribben JG, Boussiotis VA et al. Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. Blood 84(10):3261-3282, 1994.

Appendix no. I

Bearman* criteria for toxicity grading

	Grade I	Grade II	Grade III
Cardiac	Mild EKG abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms	Moderate EKG abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics	Severe EKG abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention; or decrease in voltage by more than 50%
Bladder	Macroscopic hematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedure
Renal	Increase in creatinine up to twice the baseline value (usually the last recorded before start of conditioning)	Increase in creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Pulmonary	Dypsnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO2 (>10% from baseline) but not requiring mechanical ventilation or > 50% O2 on mask and not caused by infection or CHF	Interstitial changes requiring mechanical ventilatory support or >50% oxygen on mask and not caused by infection or CHF
Hepatic	Mild hepatic dysfunction with bili > 2.0 mg% but < 6.0 mg %; or weight gain > 2.5 % and < 5 % from baseline of noncardiac origin; or SGOT increase more than 2-fold but less than 5-fold from lowest preconditioning	Moderate hepatic dysfunction with bili >6 mg% < 20 mg %; or SGOT increase with > 5-fold from preconditioning; or clinical ascites or image documented ascites > 100ml; or weight gain > 5% from baseline of noncardiac origin	Severe hepatic dysfunction with bili > 20 mg %; or hepatic encephalopathy; or ascites compromising respiratory function
CNS	Somnolence but the patient easily arousable and oriented after arousal	Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection	Seizures or coma not explained by other medication, CNS infection, or bleeding
Stomatitis	Pain and/or ulceration not requiring a continuous IV narcotic drug	Pain and/or ulceration requiring a continuous IV narcotic drug	Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation
GI	Watery stools > 500 ml but < 2,000 ml every day not related to infection	Watery stools > 2,000 ml every day not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion

NOTE: Grade IV regimen-related toxicity is defined as fatal toxicity

*Bearman Sl et al. Regimen Related Toxicity in Patients Undergoing Bone Marrow Transplantation. JCO 1988, 6(10); 1562-15

Appendix no. II

CRITERIA FOR ACUTE GRAFT-VS-HOST DISEASE

Clinical staging of acute graft-vs.-host disease according to organ involvement

STAGE	SKIN	LIVER	INTESTINAL TRACT
0	No rash	Bilirubin < 2.0 mg/dL < 34µmol/L	Diarrhea 500 ml/day
+	Maculopapular rash <25% of body surface	Bilirubin 2-2.9 mg/dL 34-50 µmol/L	Diarrhea 500-1000 ml/day
++	Maculopapular rash 25-50% of body surface	Bilirubin 3.0-6.0 mg/dL 51-102 µmol/L	Diarrhea 1000-1500 ml/day
+++	> 50% body surface	Bilirubin 6.1-15 mg/dL 103-255 µmol/L	Diarrhea 1500 ml/day
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dL > 255 µmol/L	Severe abdominal pain with or without ileus

Clinical grading of severity of acute graft-vs-host disease

GRADE	DEGREE OF ORGAN INVOLVEMENT
I	+ to ++skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	+ to +++ skin rash; + gut involvement or + liver involvement (or both); mild decrease in clinical performance
III	++ to +++ skin rash; ++ to +++ gut involvement or ++ to ++++ liver involvement (or both) marked decrease in clinical performance
IV	Similar to Grade II with ++ to ++++ organ involvement and extreme decrease in clinical performance

Source: Thomas et al, N Engl. J Med. 1975; 292, 832

Appendix no. III

Clinical Grading of Chronic GVHD

Limited Chronic GVHD:

1. Localized skin involvement,

and/or

2. Hepatic dysfunction due to chronic GVHD.

Extensive Chronic GVHD:

1. Generalized skin involvement, or
2. Localized skin involvement and/or hepatic dysfunction due to chronic GVHD

Plus

3a. Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, or

3b. Involvement of eye (Schirmer's test with less than 5 mm wetting), or

3c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or

3d. Involvement of any other target organ.

Appendix no. IV

Karnofsky Performance Status (KPS)

KPS	DEFINITION
100	Normal; no complaints; no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some sign or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most personal needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalization is indicated, although death not imminent
20	Very sick; hospitalization necessary; active support treatment is necessary
10	Moribund; fatal processes progressing rapidly
0	Dead