

**UNIVERSITY OF MINNESOTA BONE MARROW TRANSPLANT PROGRAM**

**USE OF CYCLOPHOSPHAMIDE/FLUDARABINE TO PROMOTE IN VIVO EXPANSION OF  
DONOR LYMPHOCYTE INFUSIONS (DLI) TO ENHANCE EFFICACY AFTER RELAPSE  
FROM ALLOGENEIC TRANSPLANT**

**MT2003-15**

**Original 8/4/03**

**Revised 2/23/04**

**Revised 5/18/2004**

**Revised 7/30/2004**

**Revised 12/22/2004**

**Updated 3/4/2005 (covering 1/4/05, 1/18/05, 2/11/05 revision letters)**

**Revised 3/22/2005**

**Revised 4/22/2006**

**Clarification: 10/10/2008**

**Study Committee**

Jeffrey Miller, M.D., Principal Investigator

Daniel Weisdorf, M.D.

Linda Burns, M.D.

Bruce Blazar, M.D.

Michael Verneris, M.D.

Todd DeFor, M.S.

This document contains confidential information that is the property of the Blood and Marrow Transplant Program. Do not copy, disclose, or circulate without written authorization from the Fairview-University Blood and Marrow Transplant Services Medical Director

## PROTOCOL REVISION HISTORY

Version Date	Amendment	Details of changes	Consent change?
8/4/03	original		
2/23/04		revised per CPRC review	
5/18/2004		Induction chemotherapy change: moved cyclophosphamide dose from day -7 to day -5	Yes
7/30/2004		Section 4.1.5.2 change in LFT eligibility	No
12/22/2004		Section 6.5.2 Change in method of DLI infusion. -- approved 1/20/2005	No
1/4/2005		Section 4.1.6 and 4.2.2, Appendix 1 changed eligibility to allow for subjects to be off prednisone and other immunosuppressive drugs for at least 3 days, except for subjects who are on immunosuppressive drugs for GVHD (who will continue to be excluded from the study unless they have been off the medication for 2 weeks). Approved by the IRB 1/20/2005	No
1/18/2005		Section 12.4 defined Marrow aplasia, clarified monitoring guidelines of early death. CPRC approval 2/5/05; IRB approval 3/10/2005	No
2/11/2005		Section 4.1.2, Appendix 1 Clarified eligibility of CML patients. IRB approval 2/23/2005	No
3/22/2005		Section 4.1.1.2 Clarified definition of relapse Section 7.1.1, Appendix 2. Pre-transplant bone marrow biopsies can be done within 30 days of starting therapy (rather than 10). Section 7.2.2, Appendix 2. Clarified that daily assessment of CBC will not be necessary for outpatients.	
4/22/2006		Decrease in DLI cell dose due to excess rate and intensity of GVHD	Yes
10/10/2008		Section 4.2.3 clarified that exclusion criteria is for <b>known</b> active CNS leukemia; section 13.2 revised instructions on lab draws	No

## TABLE OF CONTENTS

Schema		4
1.0	PURPOSE	5
2.0	OBJECTIVES	5
3.0	BACKGROUND	5
4.0	SELECTION OF PATIENTS	9
5.0	REGISTRATION PROCEDURES	11
6.0	TREATMENT PLAN	11
7.0	STUDY PARAMETERS	13
8.0	ADVERSE EVENT REPORTING	15
9.0	DATA AND SAFETY MONITORING PLAN	15
10.0	MEASUREMENT OF EFFECT	16
11.0	DRUG FORMULATION AND PROCUREMENT	16
12.0	EXPERIMENTAL DESIGN & STATISTICAL CONSIDERATIONS	18
13.0	CORRELATIVE STUDIES	20
14.0	RECORDS TO BE KEPT	20
15.0	REFERENCES	20
Appendix 1	ELIGIBILITY CHECKLIST	25
Appendix 2	PARAMETERS	28
Appendix 3	EXPECTED TOXICITIES, SERIOUS ADVERSE EVENTS, AND COMPLICATIONS OF THERAPY	29
Appendix 4	ACUTE GVHD ASSESSMENT GRADING SCALES	31
Appendix 5	CHRONIC GVHD CLASSIFICATION	32
Appendix 6	UNIVERSITY OF MINNESOTA ACUTE GVHD GRADING	33
Appendix 7	RESPONSE CRITERIA	34

### PI CONTACT INFORMATION:

Jeffrey S. Miller, M.D.

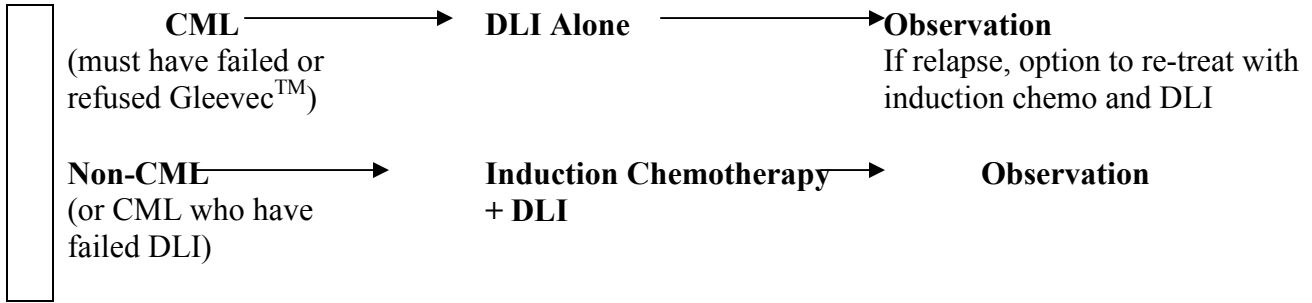
University of Minnesota Cancer Center, Room 560F

E-mail: mille011@tc.umn.edu

Phone: 612-625-7409 Fax: 612-626-4915

Hospital Operator: 612-273-3000 beeper # 7128

**SCHEMA**



**INDUCTION CHEMOTHERAPY:** (non-CML or CML who failed DLI alone)

Day	Drug
-6	fludarabine 25 mg/m <sup>2</sup> IV
-5	fludarabine 25 mg/m <sup>2</sup> IV cyclophosphamide 60 mg/kg IV
-4	fludarabine 25 mg/m <sup>2</sup> IV
-3	fludarabine 25 mg/m <sup>2</sup> IV
-2	fludarabine 25 mg/m <sup>2</sup> IV
-1	rest
0	<b>DLI</b>

**DONOR LYMPHOCYTE INFUSION (DLI) :** (all patients)

**day 0:** collect donor lymphocytes the morning of the infusion

donor cells will be infused over 2 hours at a cell dose of 0.5 x 10<sup>8</sup> CD3+ T-cells/kg

## 1.0 PURPOSE

We have considerable experience giving donor lymphocyte infusions (DLI) to patients who relapse after allogeneic transplantation. For patients with CML, DLI without chemotherapy has been very successful with durable responses in approximately 75% of patients. Unfortunately, for non-CML patients (most with acute leukemia or MDS), long-term disease free survival is significantly worse. Our conclusion from these studies is that DLI alone in non-CML patients is inadequate therapy and further modifications are needed to enhance the immune properties of DLI. Recently, animal studies and at least one human clinical trial show that adoptively transferred lymphocytes will expand in vivo if given with a regimen that clears lymphoid space (i.e. must be potent enough to induce lymphopenia). We hypothesize that the limited efficacy of DLI in non-CML patients may be enhanced by giving DLI along with a regimen that induces lymphopenia. The purpose of this study is to test the safety and efficacy of cyclophosphamide/fludarabine to enhance DLI. There are no perfect measures to evaluate in vivo expansion of lymphocytes because patients at the time of relapse often have endogenous donor lymphocytes still circulating. Patients who receive DLI + chemotherapy will be compared to historical outcomes outlined in section 3.4.

## 2.0 OBJECTIVES

- 2.1 To test whether cyclophosphamide/fludarabine, a preparative regimen thought to allow in vivo expansion of lymphocytes, is safe when combined with DLI in non-CML patients or CML patients who fail DLI without chemotherapy.
- 2.2 To test whether cyclophosphamide/fludarabine enhances the efficacy of DLI.
- 2.3 We will monitor for in vivo expansion using surrogate markers of proliferation and activation on blood lymphocytes. The validity of this analysis to detect in vivo expansion is unknown and will NOT serve as an endpoint for this trial.

## 3.0 BACKGROUND

### 3.1 Introduction

Allogeneic bone marrow transplantation results in an approximately 40-60% disease-free survival in patients with acute or chronic leukemias and myelodysplastic syndromes. The effectiveness of allogeneic bone marrow transplantation depends in part on ablation of the malignant clone with a preparative regimen and reinfusion of benign stem cells to restore normal hematopoiesis. However, it is well established that the success of allogeneic transplantation is in part mediated by a graft-versus-leukemia effect induced by donor-derived T lymphocytes or NK cells. This graft-versus-leukemia effect is usually associated with acute and/or chronic graft-versus-host disease which are also mediated by donor-derived lymphocytes. In patients with leukemia **and multiple myeloma** there is a statistically significant correlation between the occurrence of acute graft-versus-host disease and freedom from relapse (1-4).

### 3.2 Relapse after allogeneic transplantation: Past experience.

The effectiveness of bone marrow transplantation for diseases such as CML, AML, MDS and ALL depends on the disease and the disease stage at the time of transplant. Relapse rates in patients undergoing transplantation with unmodified allogeneic donor grafts vary and are approximately 15% for good risk patients and up to 50% for advanced leukemias. Relapse rates are higher in those patients who receive lymphocyte depleted grafts, especially for CML where relapse rates are as high as 70% at 3 years (1-3). CLL numbers are small and often are not separated out from other miscellaneous lymphoid malignancies in the literature, however there definite long-term survivors after allogeneic transplant. If a patient relapsed after bone marrow transplantation, prior to lymphocyte infusions, very few effective therapies were available.

In multiple myeloma, in a study of 17 patients with advanced stage disease, the rate of complete remission increased to 73% after a dose-reduced allograft increase from 18% after cytoreductive autografting. (5) For patients with AML and ALL, a second course of reinduction chemotherapy can be given with variable results. Between 12% and 70% of patients will achieve a second complete remission. However, the median survival of patients undergoing additional chemotherapy for relapse post-transplant in acute leukemia varies between 5.5 and 12 months. Long-term disease-free survival is very unlikely.

**Table 1: Treatment for Relapse Post-Transplant - AML/ALL: Chemotherapy**

Group (reference)	Number patients	CR	Median Survival
Westminster (ALL/AML)	88	7 (12%)	7 months
Minnesota (ALL) (6)	53	29 (56%)	5.5 months
EBMTR (ALL/AML) (7)	74	32 (40%)	12 months
Seattle (AML) (8)	62	20 (30%)	6 months
Seattle (ALL) (8)	94	52 (65%)	10.5 months

A second marrow transplant can be performed for AML, ALL, CML, or MDS but with significant mortality. Several studies demonstrate that continuous complete remission for greater than one year varies between 10 and 50%. A second bone marrow transplant is associated with very high early mortality (between 20 and 55%). IBMTR data evaluating second sibling transplants in 114 patients demonstrate that leukemia-free survival was 7% if undergoing transplantation within 6 months of first transplantation and 28% if second transplantation occurred greater than 6 months after the first (13). Hospitalizations for second transplants are lengthy, complicated and costly.

**Table 2: Treatment for Relapse Post-Transplant - AML/ALL/CML: Second Transplant**

Group (ref.)	Number patients	CCR (> 1 year)	Acute Mortality (< 100 days)
Minnesota	23	38%	3% <sup>9</sup>
Seattle (14)	77	15%	30%
MD Anderson	17	12%	24%
EBMTR (15)	90	12%	48%
Hopkins (16)	23	39%	---
Sidney	9	11%	44%
Duarte	5	20%	20%
Goldman (17)	16	50%	31%

### 3.3 Relapse after allogeneic transplantation: Donor Lymphocyte Infusions.

As demonstrated above, once patients relapse after allogeneic transplant, long term disease free survival is possible but limited to few patients and associated with high morbidity and mortality. Based on the premise that freedom from relapse is mediated by donor lymphocytes, reports of donor lymphocyte infusions first appeared in 1990 when three CML patients were successfully treated into cytogenetic remission with buffy coat infusions and interferon- $\alpha$ . Since then, hundreds of patients have been treated with lymphocyte infusions.

**Table 3: Treatment for Relapse Post-Transplant - AML/ALL/CML: Donor Lymphocyte Infusions**

Group (ref.)	Disease	No. patients	CR	Mortality
Goldman (18)	CML:	9 sibs/5 URD	71%	7%
	Molec/cytogen relapse	n=7	100%	0%
	Hematologic relapse	n=7	43%	14%
Arnold (19)	CML	8	75%	37%
Flomenburg (20)	CML	8	75%	13%
Antin (21)	CML	11	55%	27%
Roosnek (22)	CML	3	100%	0%
EBMTR (23)	CML	51	70%	~12%
Fay (25)	CML	6	50%	
EBMTR (23)	AML	10	40%	~12%
Henslee-Downy (24)	AML	2	50%	
Sheridan (28)	AML	4	25%	
Fay (25)	AML/ALL	13	23%	
EBMTR (23)	ALL	13	30%	~12%
Fay (25)	MM	3	66%	

### 3.4 Donor Lymphocyte Infusion Experience at the University of Minnesota since 1993.

Forty-two patients received DLI at our institution between July 1993 and December 1999. Twenty-four patients had CML, 10 had AML, 6 had MDS, 1 had ALL and 1 had juvenile myelomonocytic leukemia (JMML). All patients received T-cell replete SCT from HLA-identical siblings, except 7 patients who received their transplants from unrelated donors, and 1 patient from an HLA-mismatched sibling. Donor lymphocytes from the original donor were obtained by lymphapheresis. CML and non-CML patients received 3 lymphapheresis products, irrespective of the cell dose, but as of February 1996, all CML patients received a fixed dose of  $1 \times 10^8$  CD3<sup>+</sup> T cells/kg (n=8). Twenty five of 42 patients (60%) achieved CR after DLI. Eighteen of the 25 patients (72%) had CML, and seven patients (28%) had non-CML diseases (6 patients with AML and 1 patient with MDS). As previous studies have shown, patients treated for relapsed CML had significantly higher survival rates compared with those treated for relapsed non-CML diseases. Because of marked differences in survival, all subsequent analyses are based on the 2 groups of patients: with CML and non-CML diseases.

**Patients with CML.** Eighteen of 24 CML patients (75%) achieved molecular remission after DLI, confirmed by conversion to negative BCR/ABL status by PCR analysis. The median survival was 54 months (14-79) for patients who achieved CR, versus 6 months (2.6-35) for those who did not obtain CR after DLI ( $p < 0.01$ ). The median time to complete remission (CR) was 104 days (35-279) after DLI. Two patients (11%) relapsed 3.5 and 65 months after CR was documented. The first patient died of respiratory failure secondary to radiation pneumonitis 5 months after relapse, while the other was treated with another course of DLI, and is still alive 7 months after relapse. Eighteen patients (75%) remain alive in CR after a median follow-up of 59 months (30-79 months). CML patients had an overall probability of survival of 79% (62-96%) at 1 year, and 75% (58-92%) at 5 years after DLI. Patients who achieved remission had a 94% (82-100%) relapse-free survival 5 years after CR. All 7 CML patients (100%) treated for cytogenetic relapse only achieved CR and had a median survival of 62 months (36-76 months), while 11 of 17 patients (65%) who presented with hematological relapsed CML achieved CR and had a median survival of 38 months (14-79 months).

**Gleevec may be safer and as effective for relapsed CML.** The use of DLI as the favored first-line therapy in relapsed CML patients after allogeneic SCT has been recently challenged by the specific inhibitor of the BCR-ABL tyrosine kinase imatinib mesylate (Gleevec). Recent reports showed encouraging results, with overall response rates as high as 79%, and complete cytogenetic responses of 35% (29, 30). In the study of Kantarjian et al., imatinib mesylate was used in 28 patients, of whom 13 (46%) received DLI a median of 4 months (2-39 months) prior to imatinib. While it is likely that the responses noted in those patients (11 of 13 responded to imatinib) was due to imatinib itself, a DLI influence cannot be discarded as it is well described that responses after DLI may be protracted and occur as late as 6 to 12 months after therapy. Given the safety profile of Gleevec compared to DLI, it will be offered as first line therapy for CML patients who relapse after allogeneic transplant (may want to start at time immunosuppression being withdrawn).

**Patients with non-CML malignancies.** The overall probabilities of survival for non-CML patients were 28% (7-49%) at 1 year, and 6% (0-16%) at 5 years after DLI. Seven of 18 (39%) non-CML patients achieved complete remission. Among them, 4 patients (57%) were in remission at the time of DLI from prior treatment with salvage chemotherapy, and could not be evaluable for a direct DLI effect. Overall, the non-CML patients had a median survival of 5 months (0.6-97 months). Whereas patients who attained CR ( $n=7$ ) had a median survival of 8 months (range 2.6-97 months), those who did not achieve CR had a median survival of 2.7 months (range 0.6-24 months). Four of 11 patients (36%) pre-treated with salvage chemotherapy achieved CR prior to DLI and survived a median of 20 months (2.6-97 months). Three of 7 patients (43%) treated with DLI without prior salvage chemotherapy attained CR and had a median survival of 5 months (2.6-36 months). For the 3 evaluable patients who attained CR from direct DLI effect, remission occurred a median of 29 days (27-70 days) after DLI. The median time to relapse for patients in CR from direct DLI effect was 8 months (1-15 months) compared with 5.5 months (1.7-25 months) for patients in CR after salvage chemotherapy. Our conclusion from these studies is that DLI is inadequate for non-CML diseases, which is the major focus of this protocol.

### 3.5 Lymphoid space must be cleared for expansion of adoptively transferred lymphocytes.

Growing evidence suggests that preparative therapy must be strong enough to induce lymphopenia if adoptively transferred lymphocytes are to expand in vivo. The hypothesis is that lymphopenia (or clearing space) changes the competitive balance between transferred lymphocytes and endogenous lymphocytes.



Alternatively, lymphopenia induces survival factors or depletes inhibitory effects (cells or soluble factors) (reviewed in 31, 32). In recent murine studies, preparative regimens sufficient to induce lymphopenia allow homeostatic T-cell expansion in vivo and can potentiate effective antitumor immunity (33). This concept has recently been tested in human clinical trials at the NIH by Rosenberg's group (34). T-cell lymphopenia was induced by cyclophosphamide (60 mg/kg/day x 2 followed by fludarabine 25 mg/m<sup>2</sup>/day x 5 days). This therapy allowed in vivo expansion of adoptively transferred cytotoxic T-lymphocytes with specificity for melanoma cells, resulting in subsequent clinical efficacy. This trial is the basis for the preparative regimen to be tested here. We have elected to only give 1 dose of cyclophosphamide since patients are already heavily pretreated and to proceed cautiously. Both drugs used in this regimen, cyclophosphamide and fludarabine, have demonstrated activity against hematologic malignancies (35-38) and should result in lymphopenia, and hence facilitate in vivo expansion of lymphocytes as outlined here.

## 4.0 SELECTION OF PATIENTS

### 4.1 Inclusion criteria

- 4.1.1 Patients (age  $\geq$  1 years) with a diagnosis of relapse after related or unrelated allogeneic stem cell transplantation for a hematological malignancy.
  - 4.1.1.1 For CML, relapse will be defined as any cytogenetic evidence of a Philadelphia chromosome or persistence of BCR/ABL rearrangements by molecular testing on at least two measurements over a 6 month interval. If cytogenetics are normal and there is PCR evidence of a BCR/ABL fusion, patients will be eligible if they have evidence of a quantitative increase in CML measured either by quantitative PCR or by fluorescent in situ hybridization (FISH).
  - 4.1.1.2 For non-CML, relapse will be defined based on disease specific morphologic criteria from a bone marrow biopsy and aspirate or recurrence of disease specific cytogenetics. For disease specific definition of relapse, see appendix 3. Relapse can be determined morphologically with less than 5 percent blasts if definitive relapse can be determined. Equivocal results for relapse should result in a repeated test after an appropriate time interval (suggested 1 month) to determine eligibility.  
Post-transplant **lymphoproliferative diseases** (often referred to as EBV-associated lymphomas) are NOT eligible for this protocol.
- 4.1.2 For Chronic Phase CML patients only
  - 4.1.2.1 must have failed (no response in 3 months or incomplete response at 6 months) or refused treatment with Gleevec
  - 4.1.2.2 if no prior DLI, CML patients will first have DLI– if relapse occurs after DLI, DLI with chemotherapy per this protocol will be offered
- 4.1.3 Patients must be within one year of identification of relapse **or if beyond that time period, must have at least 10% donor DNA by RFLP or cytogenetics.**
- 4.1.4 Same allogeneic donor (sibling or URD) used for transplantation is available for lymphocyte donation.
- 4.1.5 No severe organ damage (by laboratory or clinical assessment) as measured by:
  - 4.1.5.1 blood creatinine  $\leq$  2.0 mg/dL
  - 4.1.5.2 liver function tests  $<$  5 x normal
  - 4.1.5.3 left ventricular ejection fraction  $>$  40% (testing required only if symptomatic or prior known impairment).

- 4.1.5.4 pulmonary functions > 50% (testing required only if symptomatic or prior known impairment). Oxygen saturation (>92%) can be used in child where PFT's cannot be obtained.
- 4.1.5.5 chest x-ray without evidence of active infection
- 4.1.6 Off prednisone and other immunosuppressive agents (given for any reason) for at least 3 days prior to DLI infusions.
- 4.1.7 Performance status  $\geq$  60%
- 4.1.8 Women must not be pregnant or lactating. The agents used in this study may be teratogenic to a fetus and there is no information on the excretion of agents into breast milk All females of childbearing potential must have a blood test or urine study within 2 weeks prior to registration to rule out pregnancy
- 4.1.9 Women of childbearing potential and sexually active males are strongly advised to use an accepted and effective method of contraception
- 4.1.10 Patient must given written informed consent indicating understanding of the nature of the treatment and its potential risks

## **4.2 Exclusion criteria**

- 4.2.1 Concurrent signs of acute or chronic graft-versus-host disease requiring ongoing treatment at the time of relapse will be ineligible.
- 4.2.2 Patients being treated for GVHD with prednisone, cyclosporine, Imuran or other immunosuppressive medications are not eligible until these medications are discontinued for at least 2 weeks without a flare of GVHD.
- 4.2.3 Known active CNS leukemia
- 4.2.4 Active fungal infection or pulmonary infiltrates (stable prior treated disease is allowable)
- 4.2.5 HIV positive

## **4.3 2<sup>nd</sup> step eligibility – CML patients who relapsed after DLI alone**

- 4.3.1 diagnosis of relapse after DLI
- 4.3.2 meets all inclusion and exclusion criteria in sections 4.1 and 4.2

## **4.4 Donor Eligibility**

- 4.4.1 Able to undergo lymphapheresis - donor's weighing less than 40 kg. (children) will need evaluation by a pediatrician to evaluate for suitability of the lymphapheresis procedure
- 4.4.2 HIV-1, HIV-2 negative, HTLV-1, HTLV-2 negative, Hepatitis B and C negative, not pregnant and good general medical condition.
- 4.4.3 Able to give informed written consent

## **5.0 REGISTRATION PROCEDURES**

### **5.1 Registration with the Clinical Trials Office (CTO)**

All patients should be registered with the CTO in addition to other registration procedures required by the protocol.

CML patients who are treated with DLI alone on this protocol and relapse should be re-registered for this protocol.

To register a patient to this study, complete the Subject Registration Form and study specific eligibility checklist (appendix 1) and fax it to the Clinical Trials Office Registrar at (612) 624-9654. To register by phone call (612) 624-9487 with the completed Subject Registration Form available.

**5.2 Patients who are registered and do not begin study treatment**

If a patient is registered to the study, and is later found not able to begin the planned study treatment, for whatever reason, the patient will be removed from study and treated at the physician’s discretion. Study data will be collected until the time of off study. The reason for removal from study will be clearly indicated on the case report forms.

If a patient begins treatment, and then is discontinued for whatever reason, the patient must be followed per section 7.0.

**6.0 TREATMENT PLAN**

**6.1 At diagnosis of relapse**

At the time of relapse, patients should be tapered off immunosuppressive medications as soon as possible.

It is unknown whether Gleevec alters the response to or toxicity from DLI. Since Gleevec is active in BCR/ABL positive malignancies and not known to be immunosuppressive, concomitant Gleevec will be the allowed in patients who receive DLI for a BCR/ABL positive leukemia.

**6.2 Before Day –6**

Determine patient and donor eligibility.

Obtain donor and recipient consent.

Evaluate donor venous access, donor blood for CBC, Hepatitis B, C and HIV-1, HIV-2, HTLV-1, HTLV-2, serologic test for syphilis, ALT, EBV and CMV serology.

Recipient Disease staging as in section 7.0

**6.3 Day -6**

Admit, begin allopurinol (day -6 through day 0), insert venous access device

**6.4 Day –6 through day –2 - chemotherapy administration (Non-CML and CML who have relapsed after DLI alone):**

Obese patients should be dosed half way between ideal and actual weights

Days –6	Fludarabine 25 mg/m <sup>2</sup> IV over 30 minutes
Days –5	Fludarabine 25 mg/m <sup>2</sup> Cyclophosphamide 60mg/kg IV over 2 hours Mesna 12mg/kg (pre and 3, 6, 9, and 12 hours after cyclophosphamide)  Vigorous intravenous hydration (2000-3000 ml/m <sup>2</sup> /day) should be given from 6 hours prior to the cyclophosphamide

	dose and continuing for 24 hours after the end of the cyclophosphamide infusion. Adequate diuretics should be given and patients urged to urinate every 1-2 hours to ensure urinary output of at least 200 ml every 2 hours to maintain appropriate polyuria and fluid balance. Patients should be weighed BID during cyclophosphamide administration to aid in managing fluid balance.
Days -4	Fludarabine 25 mg/m <sup>2</sup>
Days -3	Fludarabine 25 mg/m <sup>2</sup>
Days -2	Fludarabine 25 mg/m <sup>2</sup>
Day -1	Rest day

## 6.5 Day 0 – donor lymphocyte infusion (all patients)

### 6.5.1 Lymphapheresis

Donor lymphocytes obtained by lymphapheresis should be obtained from the same allogeneic donor (sibling or unrelated donor) used for transplantation. For local related donors, lymphocyte collections will be performed on the morning of the planned infusion using standard automated mononuclear cell collection techniques by the University of Minnesota Blood Bank. For unrelated donors or non-local related donors, the lymphocyte collects will be performed at an outside facility according to procedures set by the National Marrow Donor Program. If the collection is non-local, coordination for the timing of the cell collection and the ability to transport the cells to the University of Minnesota is required.

### 6.5.2 Donor Lymphocyte infusion

All patients will receive a cell dose of  $0.5 \times 10^8$  CD3+ T-cells/kg.

The lymphapheresis product will be infused without concurrent GVHD prophylaxis therapy. Donor lymphocytes will be infused over 2 hours. Patients receiving infusions on an outpatient basis will be monitored for at least 2 hours for acute transfusion related complications.

## 6.6 Dose Modifications

There are no dose modifications for this protocol except after discussion with the principal investigator and based on modifications judged to be in the patient's best interest. For example, there is some rationale for giving a lower dose of DLI in patients who relapse after T-cell depleted transplants. Since this is unusual in our current cohorts and dose modifications would be an unusual occurrence.

## 6.7 Supportive Care

6.7.1 All supportive measures consistent with optimal patient care will be given throughout the study.

6.7.2 During infusions, patients will be monitored for occurrence of untoward effects of the infusion of allogeneic lymphocytes such as rash, acute allergic reaction, bronchospasm, respiratory distress, acute vascular leak syndrome, localized or systemic infections.

- 6.7.3 Since G-CSF may have immunosuppressive effects on lymphocytes, its use should be avoided if possible. However, in the setting of severe neutropenia (ANC < 500), unexplained fever or documented infection, use of G-CSF will be at the discretion of the caring physician.
- 6.7.4 Steroids should not be used as anti-emetics under any circumstances during this protocol.

## **6.8 Duration of Therapy**

Patients will receive protocol therapy unless:

- 6.8.1 Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued.
- 6.8.2 Patient withdraws consent.

## **6.9 Duration of Follow-up**

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed for 6 months regardless of therapy. Follow-up for response and survival will continue for at least 5 years from the date of registration.

## **7.0 STUDY PARAMETERS**

### **7.1 Required observations (before starting treatment)**

#### 7.1.1 Clinical Observations

- A complete pre-infusion history and physical exam as well as laboratory tests to confirm eligibility (within 30 days of initiating therapy).
- CBC, differential, platelet count  
electrolytes, BUN, blood creatinine, blood glucose  
AST, ALT, bilirubin, alkaline phosphatase
- Chest X-ray
- MUGA and/or PFT's if clinically indicated
- bone marrow aspirate **and biopsy** - for the presence of donor or host derived cell populations (RFLP, chromosome studies) and leukemia by routine morphologic analysis and other studies as indicated (i.e. BCR/ABL, Ph chromosome or other chromosome abnormalities).

7.1.2 Research samples – see section 13.1

### **7.2 Required observations (during and after treatment)**

#### 7.2.1 Clinical observations

An interim assessment will be performed daily until neutrophil recovery including days +14, +21, then 1, 2, 3 and 6 months post, then as clinically indicated.

Patients will be monitored after infusion of allogeneic cells for the occurrence of acute and chronic graft-versus-host disease, which will be staged according to the current GVHD protocol through the University of Minnesota BMT Program.

7.2.2 Clinical labs (minimal requirements, additional tests as clinically indicated)

CBC, differential, platelet count: daily until neutrophil recovery, including +14, +21, then 1, 2 and 3 months post. Daily assessment of neutrophil recovery is not necessary if the patient is an outpatient. Follow up for this parameter will be performed as clinically indicated. Typically this would be every one to four days until neutrophil recovery.

electrolytes, BUN, blood creatinine, blood glucose: QOD until neutrophil recovery, including +14, +21, then 1, 2 and 3 months post.

AST, ALT, bilirubin, alkaline phosphatase: days 0, +7, +14, +21, then 1, 2 and 3 months post.

A bone marrow aspirate and biopsy for the presence of donor or host derived cell populations (RFLP, chromosome studies) and leukemia by routine morphologic analysis and other studies as indicated (i.e. BCR/ABL, Ph chromosome or other chromosome abnormalities) will be performed 1 and 3 months after therapy.

Each lymphocyte collection will be analyzed for cell count, as well as T-cells, T-subsets, NK cells, and other mononuclear cells (CD3, CD4, CD8, CD16, CD56 called a T<sub>H</sub>/T<sub>S</sub> panel to be performed by the immunophenotyping lab).

#### 7.2.3 Research samples – see section 13.2

### **7.3 Follow-up after 3 months**

Follow-up for response, toxicity, and survival will occur at 6 months and 1 year after the infusion and then annually for at least 5 years from study enrollment.

Since most patients with poor-prognosis myeloid leukemia are expected to die of their disease, beyond 3 months, deaths due to additional therapy (such as transplant for example) or relapse will be reported to the IRB and the CPRC as part of annual reporting.

## **8.0 ADVERSE EVENT REPORTING**

All subjects will be monitored continuously for all serious unexpected and selected serious adverse experiences during the first 3 months after the donor lymphocyte infusion. Afterward, subjects will be monitored at 6 months and 1 year after the infusion and then annually thereafter. Serious adverse events include: graft failure/autologous recovery, severe acute GVHD (Grades III and IV), relapse, and death. Serious adverse events will be reported within 10 working days of the event or knowledge of the event during the first 100 days. After day 100, only death or a serious unexpected adverse event will be reported 10 working days of knowledge of the event. All other adverse events will be reported annually.

Serious adverse events will be reported simultaneously to the Institutional Review Board and Cancer Protocol Review Committee/Data Safety and Monitoring Council as required. Toxicities, complications of therapy and other adverse events, which are considered expected as part of therapy in general, are listed in

the Appendix 3: Expected Transplant Related Toxicities. Other toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 3.0 (CTCAE)

### **Reporting of Other Second Primary Cancers**

All cases of new primary cancers that occur on protocols monitored by the University of Minnesota Cancer Center Clinical Trial Office during or after protocol must be reported to CTO, according to the follow-up schedule outlined in the protocol (on the Second Primary Cancer Form) within 30 days of diagnosis, regardless of relationship to protocol treatment.

Once data regarding survival and remission status are no longer required by the protocol, only second primaries thought to be possibly related to protocol treatment should be reported. A copy of the pathology report should be sent, if available.

## **9.0 DATA AND SAFETY MONITORING PLAN**

This study will be in compliance with the University of Minnesota Cancer Center's Data & Safety Monitoring Plan, which can be accessed at <http://www.cancer.umn.edu/page/resource/dataplan.html>

Regular meetings of the study's principal investigator and staff will be held to discuss matters related to the safety of protocol participants (SAE reporting), validity and integrity of the data, enrollment rate, retention of participants, adherence to protocol, and data completeness

The Principal Investigator will provide at least monthly monitoring of patient safety with quarterly reporting to the Clinical Trials Office (CTO) for distribution to the Cancer Protocol Review Committee's (CPRC) Data & Safety Monitoring Council (DSMC).

At the time of the IRB continuing review, the Principal Investigator will report to the CPRC the number of patients entered on the trial, the number of patients treated, a summary of all adverse events reported to date using CTC grading, a specific list of serious adverse events requiring immediate reporting, and significant literature reporting developments that may affect the safety of participants or the ethics of the study.

## **10.0 MEASUREMENT OF EFFECT**

See appendix B for disease specific response criteria.

## **11.0 DRUG FORMULATION AND PROCUREMENT**

### **11.1 Cyclophosphamide**

11.1.1 Other names: Cytoxan, Neosar, CTX, CPM

11.1.2 Classification: alkylating agent

11.1.3 Mode of Action: Inactive in its parent form; Activated by the liver cytochrome P450 microsomal system to the cytotoxic metabolites phosphoramidate mustard and acrolein; Cyclophosphamide metabolites form cross links with DNA resulting in inhibition of DNA synthesis and function; Cell cycle-nonspecific agent, active in all phases of the cell cycle

- 11.1.4 Storage and Stability: Injectable powder is stored at room temperature. Reconstituted solution is stable for 24 hours room temperature and for 6 days upon refrigeration.
- 11.1.5 Dose Specifics: In this study, cyclophosphamide will be given as a single intravenous dose. The dose will be 60 mg/kg with obese patients dosed half way between ideal and actual weights.
- 11.1.6 Preparation: Dilute vials with sterile water. It is important to shake well so that the solution is completely dissolved.
- 11.1.7 Route of Administration: Intravenously
- 11.1.8 Incompatibilities--drug interactions: Phenobarbital, phenytoin, and other drugs that stimulate the liver P450 system, anticoagulants, digoxin, doxorubicin special considerations: use with caution in patients with abnormal renal function, IV hydration required for high dose administration, pregnancy category D
- 11.1.9 Availability commercially available
- 11.1.10 Side Effects
- myelosuppression
  - bladder toxicity
  - nausea and vomiting
  - alopecia
  - amenorrhea with ovarian failure
  - cardiotoxicity with high doses
  - increased risk of secondary malignancies
  - immunosuppression
- inappropriate secretion of antidiuretic hormone (SIADH)  
hypersensitivity reaction
- 11.1.11 Nursing Implications
- monitor CBC, platelet count
  - advise patient of possible alopecia
  - assess hydration and fluid balance
  - premedicate with antiemetics
  - observe for possible phlebitis
  - administer antiemetics as indicated

## 11.2 Fludarabine

11.2.1 Other names: 2-Fluoro-ara-AMP, Fludara

11.2.2 Classification: antimetabolite



### 11.2.3 Mode of Action:

- 5-Monophosphate analog of arabinofuranosyladenosine (ara-A) with high specificity for lymphoid cells. Presence of the 2-fluoro group on adenine ring renders fludarabine resistant to breakdown by adenosine deaminase
- Considered a prodrug
- Antitumor activity against both dividing and resting cells
- Triphosphate metabolite incorporates into DNA resulting in inhibition of DNA chain extension
- Induction of apoptosis

11.2.4 Storage and Stability: Unreconstituted drug vials are stored at room temperature.

11.2.5 Dose Specifics: In this study, fludarabine will be given at the dose of 25 mg/m<sup>2</sup> for 5 days.

### 11.2.6 Preparation

- Available in a 50 mg vial, lyophilized cake for IV use.
- Add 2 ml sterile water to vial to give a final concentration of 25 mg/mL
- May be further diluted in 100 mL of 5% dextrose or 0.9% sodium chloride
- Once reconstituted, the drug should be used within 8 hours

11.2.7 Availability: commercially available

### 11.2.8 Incompatibilities

**drug interactions: cytarabine** – fludarabine may enhance the anti-tumor activity of cytarabine by inducing the expression of deoxycytidine kinase

**cyclophosphamide, cisplatin, mitoxantrone** – fludarabine may enhance the anti-tumor activity of cyclophosphamide by inhibiting nucleotide excision repair mechanisms

**pentostatin** – increased incidence of fatal pulmonary toxicity when fludarabine is used in combination with pentostatin. Use of this combination is absolutely contraindicated.

**special considerations:** use with caution in patients with abnormal renal function, use with caution in elderly patients and in those with bone marrow impairment as they are at increased risk of toxicity, monitor for signs of infection, monitor for signs of tumor lysis, allopurinol may be given prior to initiation to prevent hyperuricemia, use irradiated blood products in patients requiring transfusion as transfusion associated GVD may rarely occur, pregnancy category D, breastfeeding is not recommended.

### 11.2.9 Route of Administration

Intravenously

### 11.2.10 Side Effects

- myelosuppression
- immunosuppression
- nausea and vomiting

- fever
- hypersensitivity reaction
- tumor lysis syndrome
- transient elevation in serum transaminases, clinically asymptomatic
- neurotoxicity observed usually with high doses – presents as weakness, agitation, confusion, progressive encephalopathy, cortical blindness, seizures, and/or coma. Thought to be secondary to demyelination process

#### 11.2.11 Nursing Implications

- monitor CBC, platelet count
- premedicate with antiemetics
- monitor for stomatitis
- observe for CNS toxicity
- anticipate tumor lysis syndrome in patients with bulky disease
- monitor pulmonary function

## 12.0 EXPERIMENTAL DESIGN AND STATISTICAL CONSIDERATIONS

### 12.1 Study Design and Objectives

The principal objective of this phase II study is to show efficacy and establish safety of the DLI procedure among the non-CML and CML patients. Due to low expected enrollment among the CML patients, the non-CML patient population is driving the study design. In order to show efficacy, the primary endpoint for this study is one year survival. Secondary endpoints include disease-free survival at one year, complete remission at one year, acute GVHD by day 100 and marrow aplasia.

### 12.2 Sample size

The sample size for this study will be based on trying to show a 20% improvement over the historical one year survival of 28% among the non-CML patients. Based on a type I error rate of .05 and a power of .80, a total of 47 non-CML patients will be required to show this difference.

Because the number of CML patients enrolled in this study is expected to be low, total enrollment of CML patients at the end of five years is estimated to be approximately 10. Because of the low patient numbers, an analysis of these patients will remain descriptive.

### 12.3 Statistical Analysis

The estimates of survival and disease free survival will be calculated by the Kaplan-Meier method. The secondary endpoints of complete remission, acute GVHD and aplasia will be calculated by cumulative incidence, treating death as a competing risk. Comparison of the endpoints will be completed by the log-rank test.

### 12.4 Monitoring guidelines

This study will be continuously monitored for aplasia and treatment related mortality prior to day 100. To help guide the protocol committee in evaluating the safety of the protocol, the following monitoring boundaries will be applied to the non-CML patient population.

Marrow aplasia, will be defined as an absolute neutrophil count of less than 500 with a bone marrow less than 5% cellular in the absence of disease 42 days after donor lymphocyte infusions. The monitoring boundary for marrow aplasia is 5 of 5, 6 of 12, 7 of 23, 8 of 40 or 9 of 45. This boundary has a type I error rate of .05 if the true event rate is 10% and a power of .85 for a rate of 25%.

Early death is defined as treatment related mortality (e.g. aplasia in the absence of relapse, GVHD irrespective of relapse) before day 100 after donor lymphocyte infusions. The monitoring boundary for early death is 3 of 3, 4 of 14, or 5 of 40. This boundary has a type I error rate of .05 if the true event rate is 5% and a power of .90 for a rate of 20% (1).

Due to the low rate of enrollment among the CML patients, monthly monitoring as specified in section 9.0 will be sufficient to evaluate safety among these patients. No continuous guidelines will be used.

## **12.5 Data Collection**

Standard data will be prospectively collected and recorded on the BMT research database, including the adverse events. Patients will be followed for complications and death according to standard procedures.

Goldman AI, Hannan PJ. Optimal continuous sequential boundaries for monitoring toxicity in clinical trials, a restricted search algorithm. *Statistics in Medicine* 20:11 (2001).

## **12.6 Gender and ethnicity statement**

This study is open to men and women of all racial/ethnic groups. The patient enrollment pattern is expected to be similar to that of other hematologic cancer studies. It is not anticipated that the outcome will be affected by race. The study will not have separate accrual targets for different subgroups.

## **13.0 CORRELATIVE STUDIES**

### **13.1 Pre-treatment:**

A sample of bone marrow will be obtained pre-treatment at the time of clinical sampling if possible (specimen need not be re-drawn if for research use only). Approximately 30 cc of a heparinized sample will be requested. Pediatric patient research samples (blood or marrow) will be 20 cc or a maximum of 2 cc/kg.

For all patients: 80 ml of heparinized blood (8 green top tubes) and 10 ml of serum (red top tubes) prior to treatment (day -6 or before) will be sent to Translational Therapy Lab for phenotype and functional analyses (Call 612-625-6165 to pick up sample). Pediatric patient blood draws will be 20 cc of heparinized blood (2 green top tubes) or a maximum of 2 cc/kg.

### **13.2 During and after treatment:**

80 ml of heparinized blood (8 green top tubes) and 10ml of serum (1 red top tube) will be sent to Translational Therapy Lab for phenotype and functional analyses on days +14, +28, and 3 months post DLI

infusion.(Page 2316 for pick-up, if not in town – OK to mail). Pediatric patient blood draws will be 20 cc of heparinized blood (2 green top tubes) or a maximum of 2 cc/kg.

OK to allow  $\pm$  2 days for any scheduled time point to allow for weekends and scheduling variability

The above sample will be analyzed for:

lymphocyte subsets by immunophenotyping (including Ki67 – proliferation marker)

Pediatric patient research samples (blood or marrow) will be 20 cc or a maximum of 2 cc/kg.

#### **14.0 RECORDS TO BE KEPT**

##### Records Retention

The investigator will retain study records, including source data, copies of CRF's, and all study correspondence indefinitely in a secured facility. In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study, with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO prior to destroying any source documents.

#### **15.0 REFERENCES**

1. Marmont AM, Horowitz MM, Gale RP et al.: T-Cell Depletion of HLA-Identical Transplants in Leukemia. *Blood* 78:2120, 1991
2. Sullivan KM, Weiden PL, Storb R et al.: Influence of Acute and Chronic Graft-Versus-Host Disease on Relapse and Survival After Bone Marrow Transplantation From HLA-Identical Siblings as Treatment of Acute and Chronic Leukemia. *Blood* 73:1720, 1989
3. Wagner JE, Zahurak M, Piantodosi S et al.: Bone Marrow Transplantation of Chronic Myelogenous Leukemia in Chronic Phase: Evaluation of Risks and Benefits. *J Clin Oncol* 10:779, 1992
4. Luft T., Goldschmidt H., Ho AD, et al: Dissociation of Putative Graft-Versus-Haematopoiesis and Graft-Versus-Myeloma Effects in Patients With Rapidly Progressive Multiple Myeloma. *Brit. J of Haematology*. 123(4): 646-53, Nov 2003.
5. Kroger N., Schwerdtfeger R., Kiehl M., et al: Autologus Stem Cell Transplantation Followed by a Dose-Reduced Allograft Induces High Complete Remission Rate in Multiple Myeloma. *Blood* 100(3): 755-60, 2002 Aug 1.
6. Bostrom B, Woods WG, Nesbit ME et al.: Successful Reinduction of Patients with Acute Lymphoblastic Leukemia who Relapse Following Bone Marrow Transplantation. *J Clin Oncol* 5:376, 1987
7. Frassoni F, Barrett AJ, Granena A et al.: Relapse After Allogeneic Bone Marrow Transplantation for Acute Leukaemia: A Survey by the E.B.M.T. of 117 Cases. *Br J Haematol* 70:317, 1988

8. Mortimer J, Blinder MA, Schulman S et al.: Relapse of Acute Leukemia After Marrow Transplantation: Natural History and Results of Subsequent Therapy. *7:50*, 1989
9. Steegmann JL, Perez M, Vazquez L et al.: Interferon Alpha Treatment of Accelerated-Phase Chronic Myeloid Leukemia in Relapse After Bone Marrow Transplantation: A Case with Complete Cytogenetic and Molecular Remission. *Bone Marrow Transplant 7:65*, 1991
10. Higano CS, Raskind WH, Singer JW: Use of Alpha Interferon for the Treatment of Relapse of Chronic Myelogenous Leukemia in Chronic Phase After Allogeneic Bone Marrow Transplantation. *Blood 80:1437*, 1992
11. Archese W, Goldman JM, D'Arcangelo E et al.: Outcome for Patients Who Relapse After Allogeneic Bone Marrow Transplantation for Chronic Myeloid Leukemia. *Blood 82:3211*, 1993
12. Nagler A, Ackerstein A, Barak V, Slavin S: Treatment of Chronic Myelogenous Leukemia with Recombinant Human Interleukin-2 and Interferon- $\alpha$ 2a. *J Hematotherapy 3:75*, 1994
13. Mrcic M, Horowitz MM, Atkinson K et al.: Second HLA-Identical Sibling Transplants for Leukemia Recurrence. *Bone Marrow Transplant 9:269*, 1992
14. Radich JP, Sanders JE, Buckner CD et al.: Second Allogeneic Marrow Transplantation for Patients with Recurrent Leukemia After Initial Transplant with Total-Body Irradiation-Containing Regimens. *J Clin Oncol 11:304*, 1993
15. Barrett AJ, Locatelli F, Treleaven JG, Gratwohl A, Szydlo R, Zwaan FE: Second Transplants for Leukaemic Relapse After Bone Marrow Transplantation: High Early Mortality but Favourable Effect of Chronic GVHD on Continued Remission. *Br J Haematol 79:567*, 1991
16. Wagner JE, Vogelsang GB, Zehnbauer BA, Griffin CA, Shah N, Santos GW: Relapse of Leukemia After Bone Marrow Transplantation: Effect of Second Myeloablative Therapy. *Bone Marrow Transplant 9:205*, 1992
17. Cullis JO, Schwarzer AP, Hughes TP et al.: Second Transplants for Patients With Chronic Myeloid Leukaemia in Relapse After Original Transplant with T-Depleted Donor Marrow: Feasibility of Using Busulphan Alone for Re-Conditioning. *Br J Haematol 80:33*, 1992
18. 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*, 1994
19. Hertenstein B, Wiesneth M, Novotny J et al.: Interferon-Alpha and Donor Buffy Coat Transfusions for Treatment of Relapsed Chronic Myeloid Leukemia After Allogeneic Bone Marrow Transplantation. *Transplantation 56:1114*, 1993

20. Drobyski WR, Keever CA, Roth MS: Salvage Immunotherapy Using Donor 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, 1993
21. 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, 1994
22. Helg C, Roux E, Beris P et al.: Adoptive Immunotherapy for Recurrent CML After BMT. *Bone Marrow Transplant* 12:125, 1993
23. Kolb HJ, de Witte T, Millermuller J et al.: Graft-Versus-Leukemia Effect of Donor Buffy Coat Transfusions on Recurrent Leukemia After Marrow Transplantation. *Blood* 82:214a, 1993
24. Pati A, Godder K, Lamb L, Gee A, Henslee-Downey PJ: Donor Leukocyte Infusions (DLI) to Treat Relapsed Acute Myeloid Leukemia (AML) Following Partially Mismatched Related Donor (PMRD) Bone Marrow Transplantation (BMT). *Blood* 84:339a, 1994
25. Collins R, Wolff S, List A et al.: Prospective Multicenter Trial of Donor Buffy Coat Infusion for Relapsed Hemtologic Malignancy Post-Allogeneic Bone Marrow Transplantation (BMT). *Blood* 84:333a, 1994
26. Kolb HJ, Schattenberg A, Goldman JM et al: Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 86:2041-50, 1995.
27. Mackinnon S, Papadopoulos EB, Carabasi MH et al: 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.
28. Szer J, Grigg AP, Phillips GL, Sheridan WP: Donor Leucocyte Infusions After Chemotherapy for Patients Relapsing With Acute Leukaemia Following Allogeneic BMT. *Bone Marrow Transplant* 11:109, 1993
29. Kantarjian HM, O'Brien S, Cortes JE, et al. Imatinib mesylate therapy for relapse after allogeneic stem cell transplantation for chronic myelogenous leukemia. *Blood* 2002, **100**: 1590-1595.
30. Olavarria E, Craddock C, Dazzi F, et al. Imatinib mesylate (STI571) in the treatment of relapse of chronic myeloid leukemia after allogeneic stem cell transplantation. *Blood* 2002; **99**: 3861-3862.
31. Theofilopoulos AN, Dummer W, Kono DH. T cell homeostasis and systemic autoimmunity. *J. Clin. Invest.* 2001;108(3):335-40.
32. Maine GN, Mule JJ. Making room for T cells. *J. Clin. Invest.* 2002; 110(2):157-59.

33. Dummer W, Niethammer AG, Baccala R, Lawson BR, Wagner N, Reisfeld RA, Theofilopoulos AN. T cell homeostatic proliferation elicits effective antitumor autoimmunity. *J. Clin. Invest.* 2002;110(2):185-92.
34. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robins MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA. *Science.* 2002;298:850-54.
35. Kornblau SM, Kantarjian H, O'Brien S, Andreeff M, Koller CA, Beran M, Keating M, Estey E. CECA-cyclophosphamide, etoposide, carboplatin and cytosine arabinoside--a new salvage regimen for relapsed or refractory acute myelogenous leukemia. *Leukemia & Lymphoma.* 1998;28(3-4):371-75.
36. Quintino CM, Masoudi B, Carrara S, Cerri R, Pierri I, Canepa L, Miglino M, Muner P, Damasio E, Gobbi M. Cost of de novo acute myeloid leukemia induction therapy in adults: analysis of EORTC-GIMEMA AML10 and FLANG regimens. *J Exp. & Clin. Cancer Res.* 2001;20(2):165-73.
37. Higashi Y, Turzanski J, Pallis M, Russell NH. Contrasting in vitro effects for the combination of fludarabine, cytosine arabinoside (Ara-C) and granulocyte colony-stimulating factor (FLAG) compared with daunorubicin and Ara-C in P-glycoprotein-positive and P-glycoprotein-negative acute myeloblastic leukaemia. *British J Haematol.* 2000;111(2):565-9.
38. Koller CA, Kantarjian HM, Feldman EJ, O'Brien S, Rios MB, Estey E, Keating M. A phase I-II trial of escalating doses of mitoxantrone with fixed doses of cytarabine plus fludarabine as salvage therapy for patients with acute leukemia and the blastic phase of chronic myelogenous leukemia. *Cancer.* 1999;86(11):2246-51.

MT2003-15

**Use of cyclophosphamide/fludarabine to promote in vivo expansion of donor lymphocyte infusions (DLI) to enhance efficacy after relapse from allogeneic transplant**

**ELIGIBILITY CHECKLIST**

**INCLUSION CRITERIA (answers should be yes):**

**Circle yes or no**

**Disease Criteria**

Yes/No Diagnosis of relapse after related or unrelated allogeneic stem cell transplantation for a hematological malignancy

Yes/No/NA **For CML**, relapse will be defined as any cytogenetic evidence of a Philadelphia chromosome or persistence of BCR/ABL rearrangements by molecular testing on at least two measurements over a 6 month interval. If cytogenetics are normal and there is PCR evidence of a BCR/ABL fusion, patients will be eligible if they have evidence of a quantitative increase in CML measured either by quantitative PCR or by fluorescent in situ hybridization (FISH).

Yes/No/NA For non-CML, **relapse will be defined based on disease specific morphologic criteria from a bone marrow biopsy and aspirate or recurrence of disease specific cytogenetics. For disease specific definition of relapse, see appendix 3.**

Yes/No/NA For CML patients only: **if no prior DLI, CML patients will first have DLI without chemo (per MT 9524)**  
**If relapse occurs after DLI, DLI with chemotherapy per this protocol will be offered**

Yes/No/NA **For Chronic Phase CML patients only:** must have failed ( no response in 3 months or incomplete response in 6 months) or refused treatment with Gleevec

Yes/No Must be within one year of identification of relapse or if beyond that time period, must have at least 10% donor DNA by RFLP or cytogenetics

**Patient Health Status (e.g. organ function)**

Yes/No Age  $\geq$  1 years, Performance status  $\geq$  60%

Yes/No blood creatinine  $\leq$  2.0 mg/dL

Yes/No liver function tests  $<$  5 x normal

Yes/No left ventricular ejection fraction  $>$  40% (testing required only if symptomatic or prior known impairment).

Yes/No pulmonary functions  $>$  50% (testing required only if symptomatic or prior known impairment). Oxygen saturation ( $>$ 92%) can be used in child where PFT's cannot be obtained.

Yes/No chest x-ray without evidence of active infection



**Other Eligibility Criteria as Necessitated per Protocol**

Yes/No Same allogeneic donor (sibling or URD) used for transplantation is available for lymphocyte donation

Yes/No Off prednisone and other immunosuppressive medications (given for any reason) for at least 3 days prior to DLI infusions

**EXCLUSION CRITERIA (answers should be no):**

**Circle yes or no**

Yes/No Post-transplant **lymphoproliferative disease** (often referred to as EBV-associated lymphomas)

Yes/No Pregnant or lactating

Yes/No Concurrent signs of acute or chronic graft-versus-host disease requiring ongoing treatment at the time of relapse

Yes/No **Patients being treated for GVHD** with prednisone, cyclosporine, Imuran or other immunosuppressive medications are not eligible until these medications are discontinued for at least 2 weeks prior to DLI infusions without a flare of GVHD

Yes/No Active CNS leukemia

Yes/No Active fungal infection or pulmonary infiltrates (stable prior treated disease allowed)

Yes/No HIV positive

**Donor Inclusion Criteria (answers should be yes)** Donor Name \_\_\_\_\_

Circle yes or no

Yes/No Able to undergo lymphapheresis - donor's weighing less than 40 kg (children) will need evaluation by a pediatrician to evaluate for suitability of the lymphapheresis

Yes/No HIV-1, HIV-2 negative, HTLV-1, HTLV-2 negative, Hepatitis B and C negative

Yes/No Good medical condition, not pregnant

Yes/No Signed IRB approved informed consent

INFORMED CONSENT SIGNED (YES/NO) \_\_\_\_\_ (date).

PATIENT GIVEN COPY OF CONSENT (YES/NO) \_\_\_\_\_ (date).

\_\_\_\_\_ **Does/ Does NOT** meet all eligibility criteria.  
(patient name)

\_\_\_\_\_  
PHYSICIAN SIGNATURE

\_\_\_\_\_  
DATE

**APPENDIX 2 - PARAMETERS**

	Pre: w/in 10 days of starting therapy	Daily	QOD	Day -1	Day 0	Day +1	Day +2	Day +7	Day +14	Day +21	Day +28	2 mon	3 mon
Physical Exam and watch for toxicity	X	X <sup>1</sup>							X		X	X	X
Chest X-ray	X												
CBC, diff, plt	X	X <sup>1</sup>							X	X	X	X	X
Lytes, BUN, creat, glucose	X		X <sup>1</sup>						X	X	X	X	X
AST, ALT, bili, alk phos	X				X			X	X	X	X	X	X
Bone Marrow Biopsy and aspirate	X (w/in 30 days of starting therapy)										X		X
Research bloods: 80 ml heparinized blood (8 green top tubes) and 10 ml serum (1 red top) for Translational Therapy Lab**	X								X		X		X

\*\* Pediatric patient research samples (blood or marrow) will be 20 cc or a maximum of 2 cc/kg.

OK to allow ± 2 days for any scheduled time point to allow for weekends and scheduling variability

1 -- until neutrophil recovery; daily assessment of CBC not necessary for outpatients (should be 1-4 days as clinically indicated).

2—prior to DLI infusion

**APPENDIX 3- EXPECTED TOXICITIES, SERIOUS ADVERSE EVENTS, AND COMPLICATIONS OF THERAPY**

Cyclophosphamide –

<b><u>Common</u></b> Occurs in 21-100 people out of every 100	<b><u>Less Frequent</u></b> Occurs in 5-20 people out of every 100	<b><u>Uncommon</u></b> Occurs in ≤5 people out of every 100
Nausea/vomiting Mucositis Sterility Severe suppression of blood counts Diarrhea Fluid weight gain/edema Alopecia	Hemorrhagic cystitis	Cardiomyopathy Skin rash SIADH (Syndrome of Inappropriate Anti-diuretic Hormone)

Fludarabine -

<b><u>Common</u></b> Occurs in 21-100 people out of 100	<b><u>Less Frequent</u></b> Occurs in 5-20 people out of every 100	<b><u>Uncommon</u></b> Occurs in <5 people out of every 100
Severe suppression of blood counts Diarrhea Anorexia Mucositis Nausea/vomiting Stomatitis Osteoporosis Dysuria	Chills Fever GI bleeding Peripheral edema	Neurotoxicity Agitation and confusion Blurred vision Peripheral neuropathy Hearing loss Headache Cerebellar syndrome Blindness Coma Weakness Depression Insomnia Hemorrhagic cystitis (except in FA) Abnormal renal function test Autoimmune hemolytic anemia Deep venous thrombosis

		Aneurysms Pruritic skin rash Abnormal liver function/Liver failure Constipation Transient ischemic attack Dysphagia Myalgia Arthralgia Renal failure
--	--	--

## APPENDIX 4– ACUTE GVHD ASSESSMENT GRADING SCALES

Consensus Clinical Stage and Grade of Acute GVHD (Przepiorka *et al*, 1995)

Stage	Skin	Liver	Lower Gastrointestinal Tract	Upper Gastrointestinal Tract
1	Maculopapular rash <25% of body surface	Bilirubin 2.0 – 3.0 mg/dl	Diarrhea 500 – 1000 mL/day or 280 – 555 mL/m <sup>2</sup>	No protracted nausea and vomiting
2	Maculopapular rash 25-50% body surface	Bilirubin 3.1 – 6.0 mg/dl	Diarrhea 1000 – 1500 mL/day or 556 – 833 mL/m <sup>2</sup>	Persistent nausea, vomiting or anorexia
3	Generalized erythroderma	Bilirubin 6.1 – 15.0 mg/dl	Diarrhea >1500 mL/day or >833 mL/m <sup>2</sup>	
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dl	Severe abdominal pain, with or without ileus, or stool with frank blood or melena	

## **APPENDIX 5– CHRONIC GVHD CLASSIFICATION**

### **Limited CGVHD**

Localized skin involvement (<50% body surface area) and/or

Limited hepatic involvement (abnormal LFTS; bilirubin < 3 mg/dl)

### **Extensive CGVHD**

The presence of one or more of the following criteria may be used for the diagnosis of extensive CGVHD:

- Generalized skin involvement ( $\geq 50\%$  body surface area) (see Appendix 3)
- Liver histology consistent with involvement by CGVHD with bilirubin  $\geq 3$  mg/dl

### **Positive Schirmer's test (< 5 mm wetting)**

- Histologically-proven involvement by CGVHD of oral mucosa or salivary glands

- Lung dysfunction with bronchiolitis obliterans with no evidence of viral causation on histology.

- Gastrointestinal involvement: malabsorption and/or weight loss due to anorexia without explanation other than CGVHD

## APPENDIX 6–UNIVERSITY OF MINNESOTA ACUTE GVHD GRADING

Acute GVHD Grade	Skin Stage	Liver Stage	Lower GI Stage	Upper GI Stage
<b>I</b>	1-2	0	0	0
<b>II</b>	3	1	1	1
<b>III</b>	-	2-4	2-3	
<b>IV</b>	4	-	4	

- Each column identifies minimum criteria for organ grade.
- Each grade is based on maximum stage for each individual organ involved

## APPENDIX 7 - RESPONSE CRITERIA

### CHRONIC MYELOGENOUS LEUKEMIA (CML) RESPONSE CRITERIA

#### Complete Remission (CR)

##### Peripheral Blood Counts

Neutrophil count  $\geq 1.0 \times 10^9/L$ .

Platelet count  $\geq 100 \times 10^9/L$ .

Reduced hemoglobin concentration or hematocrit has no bearing on remission status.

**Leukemic blasts must not be present in the peripheral blood.**

##### Bone Marrow Aspirate and Biopsy

Cellularity of bone marrow biopsy must be  $> 20\%$  with maturation of all cell lines.

$< 5\%$  blasts

Auer rods must not be detectable.

Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.

Must continue  $\geq 4$  weeks.

#### Partial Remission (PR)

Requires that all of the criteria for complete remission be satisfied except that the bone marrow may contain  $> 5\%$  blasts but  $< 25\%$  blasts.

If all other criteria for CR are met, then a value of  $\leq 5\%$  blasts with Auer rods or abnormal morphology is considered a partial remission.

Must continue  $\geq 4$  weeks.

#### Cytogenetic Response

**Patients will be evaluated for the presence of a cytogenetic abnormalities both before treatment and at best response. The continued presence of abnormal clones despite hematologic and marrow complete response will constitute a cytogenetic PR. Complete absence of the abnormal clone on cytogenetic evaluation will constitute a cytogenetic CR.**

#### Hematologic Improvement (HI)

**NOTE:** This category will be used for the purpose of reporting outcome, but not for reporting response rate.

This classification documents improvement in clinical features, i.e., physical findings, symptoms, absence of transfusion requirement, without total normalization of hemogram or marrow. The criteria used were utilized in a study published by M.D. Anderson Tumor Institute (16).

Marrow:  $\geq 5\% - \leq 50\%$  Blasts and Promyelocytes.

Hemogram: Hgb  $> 9.0$  gm; neutrophils  $> 1000/mm^3$ ; platelets  $> 50,000/mm^3$  and no transfusion necessary.

Physical Exam: At least 50% reduction in organomegaly or lymphadenopathy.

#### Relapse

Relapse following complete remission is defined as:

##### Peripheral Blood Counts

Reappearance of blasts in the blood.

##### Bone Marrow Aspirate and Biopsy

Presence of  $> 5\%$  blasts, not attributable to another cause (e.g., bone marrow regeneration).

**If there are no circulating blasts and the bone marrow contains 5% to 20% blasts, then a repeat bone marrow performed  $\geq 1$  week later documenting more than 5% blasts is necessary to meet the criteria for relapse.**



Stable Disease (SD)

**No progression but no improvement in marrow or peripheral blood counts.**

Progressive Disease (PD)

Continued increase in WBC count with increasing numbers of blasts in peripheral blood and marrow.  
Distinction between hyperleukocytosis due to drug versus PD must be determined.  
Progressive decline in platelet count.  
Progressive enlargement of spleen and/or lymph nodes.  
Continuation or development of systemic symptoms, including fever and night sweats.  
Progressive deterioration in marrow with decline in degree of maturation.

ACUTE MYELOGENOUS LEUKEMIA (AML) RESPONSE CRITERIA

Complete Remission (CR)

**Requires that all of the following be present for at least 4 weeks.**

Peripheral Blood Counts

Neutrophil count  $\geq 1.0 \times 10^9$  /L.  
Platelet count  $\geq 100 \times 10^9$  /L.  
Reduced hemoglobin concentration or hematocrit has no bearing on remission status.

**Leukemic blasts must not be present in the peripheral blood.**

Bone Marrow Aspirate and Biopsy

Cellularity of bone marrow biopsy must be  $> 20\%$  with maturation of all cell lines.  
 $\leq 5\%$  blasts  
Auer rods must not be detectable.  
Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.

Partial Remission (PR)

Requires that all of the criteria for complete remission be satisfied except that the bone marrow may contain  $> 5\%$  blasts but  $< 25\%$  blasts.  
If all other criteria for CR are met, then a value of  $\leq 5\%$  blasts with Auer rods or abnormal morphology is considered a partial remission.

Relapse

**Relapse following complete remission is defined as:**

Peripheral Blood Counts

**Reappearance of blasts in the blood.**

Bone Marrow Aspirate and Biopsy

Presence of  $> 5\%$  blasts, not attributable to another cause (e.g., bone marrow regeneration).

**If there are no circulating blasts and the bone marrow contains 5% to 20% blasts, then a repeat bone marrow performed  $\geq 1$  week later documenting more than 5% blasts is necessary to meet the criteria for relapse.**

ACUTE LYMPHOCYTIC LEUKEMIA (ALL) RESPONSE CRITERIA

Complete Remission (CR)

**Requires that all of the following be present for at least 4 weeks.**

#### Peripheral Blood Counts

Neutrophil count  $\geq 1.0 \times 10^9/L$ .

Platelet count  $\geq 100 \times 10^9/L$ .

Reduced hemoglobin concentration or hematocrit has no bearing on remission status.

**Leukemic blasts must not be present in the peripheral blood.**

#### Bone Marrow Aspirate and Biopsy

Cellularity of bone marrow biopsy must be  $> 20\%$  with maturation of all cell lines.

$\leq 5\%$  blasts.

**Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.**

#### Partial Remission (PR)

**Requires that all of the criteria for complete remission be satisfied except that the bone marrow may contain  $> 5\%$  blasts but  $< 25\%$  blasts.**

#### Relapse

Relapse following complete remission is defined as:

#### Peripheral Blood Counts

Reappearance of blasts in the blood.

#### Bone Marrow Aspirate and Biopsy

Presence of  $> 5\%$  blasts, not attributable to another cause (e.g., bone marrow regeneration).

If there are no circulating blasts and the bone marrow contains 5% to 20% blasts, then a repeat bone marrow performed  $\geq 1$  week later documenting more than 5% blasts is necessary to meet the criteria for relapse.

### CHRONIC LYMPHOCYTIC LEUKEMIA RESPONSE CRITERIA

#### Assessment of Clinical Response

The major criteria for determination of the response to therapy in patients with B-CLL include physical examination and evaluation of peripheral blood and bone marrow. It is recommended that the laboratory and radiographic studies which are abnormal pre-study be repeated to document the degree of maximal response.

Complete remission requires all of the following for a period of at least 2 months:

- Absence of lymphadenopathy by physical examination and appropriate radiographic techniques.
- No hepatomegaly or splenomegaly.
- Absence of constitutional symptoms.
- Normal CBC as exhibited by:
  - Polymorphonuclear leukocytes  $\geq 1500/\mu l$ .
  - Platelets  $> 100,000/\mu l$ .
  - Hemoglobin  $> 11.0$  gm/dl (untransfused).
  - Peripheral blood lymphocytes  $\leq 4000/\mu l$ .
- One marrow aspirate and biopsy should be performed no sooner than 2 months after clinical and laboratory evidence of a CR to document that a complete remission has been achieved. The marrow sample must be at least normocellular with  $< 30\%$  of nucleated cells being lymphocytes. If it is hypocellular, a repeat determination should be made in 2 weeks. Samples are to be analyzed by a pathologist and the presence or absence of nodules noted, although not included in the current definition of CR. A patient who is in CR, but has nodules, will be considered to have nodular PR & recorded separately.

- Any other laboratory assays (e.g., quantitative immunoglobulins) will not be used currently as an index for response but will be recorded for clinical correlations.

To be considered in PR, the patient must exhibit the features in sections 1,2, and, 3 (if abnormal prior to therapy) as well as one or more of the remaining features (sections 4, 5, 6) for at least 2 months. In addition to the parameters listed below, the presence or absence of constitutional symptoms will be recorded.

- $\geq 50\%$  decrease in peripheral blood lymphocyte count from the pretreatment baseline value.
- $\geq 50\%$  reduction in lymphadenopathy.
- $\geq 50\%$  reduction in size of liver and/or spleen.
- Polymorphonuclear leukocytes  $\geq 1500/\mu\text{l}$  or 50% improvement over baseline.
- Platelets  $> 100,000/\mu\text{l}$  or 50% improvement over baseline.
- Hemoglobin  $> 11.0 \text{ gm/dl}$  or 50% improvement over baseline without transfusions.

Progressive disease (PD) will be characterized by at least one of the following:

- $\geq 50\%$  increase in the sum of the products of at least 2 lymph nodes on 2 consecutive examinations 2 weeks apart (at least 1 node must be  $\geq 2 \text{ cm}$ ). Appearance of new palpable lymph nodes.
- $\geq 50\%$  increase in the size of liver and/or spleen as determined by measurement below the respective costal margin; appearance of palpable hepatomegaly or splenomegaly which was not previously present.
- $\geq 50\%$  increase in the absolute number of circulating lymphocytes.
- In the absence of progression as defined above, the presence of a  $\geq 2 \text{ gm/dl}$  decrease in hemoglobin, or  $\geq 50\%$  decrease in platelet count and/or absolute granulocyte count will not exclude a patient from continuing on study. Bone marrow aspirate and biopsy are strongly encouraged to better define the cause of the suppressed counts.
- Transformation to a more aggressive histology (e.g. Richter's syndrome or prolymphocytic leukemia with  $>55\%$  prolymphocytes).

Patients who have not achieved a CR or a PR, or who have not exhibited findings consistent with Progressive Disease will be considered as having Stable Disease.

## NON-HODGKIN'S LYMPHOMA RESPONSE CRITERIA

### Assessment of Clinical Response

It is sufficient for patients who achieve the response parameters to proceed to the next sequential treatment stage. It is not necessary to wait 4 weeks to confirm response before proceeding to the next sequential treatment phase.

### Complete Response (CR)

Patients achieving a complete regression for  $\geq 4$  weeks of all palpable and x-ray demonstrable disease and bone marrow disease judged by unilateral iliac crest bone marrow biopsy (if initially positive) will be considered to have had a complete response.

**Lymph nodes remaining in areas of previous known disease will be considered to be uninvolved if they measure  $\leq 1.0 \times 1.0 \text{ cm}$**

Lymph nodes of larger size must be documented to be free of tumor on biopsy, or have failed to enlarge over 3 months off-therapy, if a patient is to be considered a complete responder.

If the liver and/or spleen are enlarged prior to onset of treatment, they must return to normal size. Furthermore, negative liver biopsy is required to document complete remission if a positive liver biopsy was obtained prior to initiation of induction therapy.

#### Partial Response (PR)

Patients who have not achieved a complete response, but have shown a response to therapy with a 50% or greater reduction in the sum of the products of the dimensions of the measurable lesions for  $\geq$  4 weeks will be considered to have a partial response.

**If the liver is an indicator lesion, the sum of the measurements below the right costal margin, xyphoid process, and left costal margin respectively must decrease by at least 50%.**

If the spleen was enlarged prior to therapy and measured  $<$  5 cm below the costal margin, it must return to normal size. If the spleen is measured  $\geq$  5 cm below the costal margin, it must decrease in size by  $\geq$  50%.

#### Stable Disease (SD)

**Disease which does not satisfy the criteria of the other sections (CR, PR or PD) will be categorized as no change. Stable disease is also defined by at least a 4-week period that fits this description.**

#### Progressive Disease (PD)

Progressive disease is defined as an increase in size of 25% of the sum of the products of the pretreatment measurements or appearance of new lesions.

**If the liver and/or spleen increases in size  $>$  2.0 cm in distance from the costal margin, the patient will be considered a treatment failure except, if in the view of the clinician, this finding is attributed to other causes (e.g., hematopoietic growth factors may cause splenomegaly and hepatitis may cause liver enlargement).**

#### Relapse

**Relapse is defined as the re-appearance of any clinical evidence of lymphoma in a patient who has had a CR. Relapse for partial responders is defined as progressive disease relative to disease status during the partial remission.**

#### Duration of Response

**This is measured from the documented beginning of response (CR or PR) to the time of relapse.**

#### Engraftment Parameters

Neutrophil engraftment will be monitored as the number of days to an ANC (neutrophil and band forms) of  $>$  500/mm and  $>$  1,000/mm .

**Platelet engraftment will be monitored as the number of days to a platelet count of  $>$  20,000 and  $>$  50,000 independent of platelet transfusions for at least 7 days.**

The total number of red blood cell and platelet transfusions will be monitored.

## MULTIPLE MYELOMA RESPONSE CRITERIA

Response will be evaluated using the following Myeloma Response Criteria, which have been modified to exclude references to non-secretory myeloma.

### Objective Response

Response evaluation will be based on determination of reduction in serum and/or urine M-protein (monoclonal or myeloma protein) and on improvement in measurable soft tissue plasmacytomas when present. The following table presents requirements for objective response based on pretreatment protein manifestations of disease:

<b>Serum and Urine M-Protein Criteria for Objective Response<sup>1</sup></b>			
Pretreatment M-protein in:	Serum M-protein must decrease to:	<u>AN</u> <u>D</u>	24-hr Urine Excretion of M-protein must decrease to:
Serum + Urine	≤ 50% pretreatment g/dL	<u>AN</u> <u>D</u>	≤ 50% of pretreatment g/24 hr
Serum only	< 50% pretreatment g/dL	<u>AN</u> <u>D</u>	< 150 mg/24 hours
Urine only	< 1.0 g/dL	<u>AN</u> <u>D</u>	≤ 10% pretreatment/24 hours

<sup>1</sup> If present, all of these parameters must be followed for response.

Protein criteria supersede other criteria in determining response or progressive disease (PD) unless there is clear-cut, obvious progressive disease by other criteria, e.g. plasma cell leukemia, hypercalcemia, plasmacytoma, or unequivocal bone progression. In the case of uninterpretable protein data, response data may be determined by ancillary measures.

In general, serum and urine M-protein levels should be determined by electrophoresis in preference to quantitative immunoglobulin levels alone. There are exceptions, however, in which the investigator may determine the submitted M-spike value to be unreliable as in the following instances:

- Small  $\beta$ -migrating M-proteins are contaminated by normal beta globulins that are often greater in quantity than the M-spike itself.
- Cases in which the M-spike measurement provided is difficult to interpret due to excess scatter, small M-spike, or reflecting the addition of  $\beta$  plus  $\gamma$  plus  $\beta / \gamma$  regions rather than measurement of the spike itself.

In these instances, the investigator may need to rely on the quantitative serum Ig values or recalculate the M-spike from the SPEP tracing. In sequential measures, SPEP should only be compared to SPEP values and quantitative Ig values only to quantitative Ig values.

**NOTE:** Protein requirements for response must be verified on 2 consecutive determinations separated by at least 2 weeks. Unequivocal progression of skeletal disease could constitute progression, even if protein manifestations of disease are improving if the criteria in the relapse/progression section (below) are met. Collapse of bony structure from previous disease will not constitute progression or

failure to respond. For objective response criteria to be met, there must be no new bone lesions, no increase in existing lytic lesions, no recurrence or persistence of hypercalcemia, no increase in any existing plasmacytomas and no new plasmacytomas.

### Complete Response (CR)

Patients with objective response who also have complete disappearance of an M-protein and no evidence of myeloma in the bone marrow are considered to have complete response.

To be considered CR, patients must meet the following criteria:

- Complete disappearance of myeloma protein from serum: Patients with no detectable serum M-spike by serum protein electrophoresis and with quantitative immunoglobulins within normal range (may vary according to lab) will have a repeat serum immunoelectrophoresis. If no serum M-protein detected, immunofixation of serum should be performed to confirm CR.
- Complete disappearance of myeloma protein from urine: Patients must have an appropriately concentrated urine specimen. If there is no detectable urine M-protein by urine protein electrophoresis, patients will have repeat urine immunoelectrophoresis and if negative, immunofixation of urine should be done to confirm CR.
- Bone marrow biopsy demonstrating < 3% plasma cells.
- As above, there must be no evidence of progressive disease by other parameters.

### Near Complete Response (NCR)

Patients with objective response meeting CR criteria except for 1 of the following are considered to have NCR:

- Lacking repeat bone marrow
- 3%-6% bone marrow plasma cells remaining, or
- < 3% plasma cells remaining on routine bone marrow differential count but marrow aspiration or biopsy still shows clear evidence of myeloma (sheets or clusters of malignant plasma cells).

### Partial Response (PR)

Patients meeting objective response (OR) criteria but not complete response (CR) or near complete response (NCR) criteria are considered to have a partial response (PR).

### Disease Plateau

A patient in CR, NCR, or PR will be further classified as being in plateau if the following criteria are met.

**NOTE:** Plateau is not a distinct response category.

Objective response AND

Serum and urine M-protein values must be stable (< 20% variation) or must have disappeared for a period of at least 4 weeks. For the purposes of the formula below, serum M-protein is defined as the serum M-spike as measured by serum protein electrophoresis.

To qualify as < 20% variation, M-protein measurements over at least a 4-week period must satisfy

$$(MAX - MIN) \div MAX < 0.20,$$

where max = maximum M-protein value, and min = minimum M-protein value. The difference between the maximum and minimum values observed is divided by the maximum value observed. If the result is greater than 0.20, then there has been a greater than 20% variation in the M-protein values.

Date of plateau will be date of CONFIRMATION that patient is in plateau.

Any patient who is continuing to improve by any criteria would not be considered in plateau and should remain on treatment until the continued improvement plateaus.

### No Response (NR)

Failure to meet response criteria outlined in Section 6.1.

### Relapse or Progression (PD)

Patients meeting two or more of the criteria in 1-4 OR 5 will be considered to have relapse or progression:

1. Increase in serum M-protein to > 50% above the lowest response level or a rise of 2.0 g/dL (this increase must be to a level >1.0 g/dL if it is to constitute the sole protein manifestation of relapse). (The serum M-protein should be measured by electrophoresis unless the M-spike is <1.5 g/dL. However, when only quantitative Ig values are reported, it may be accepted as definitive even though serum protein electrophoresis should have been done.)
2. Increase in urine M-protein to 50% above the lowest remission value for 24-hour excretion or an increase of 2g/24 hours of urine M-protein (this increase must also represent a 24-hour M-protein excretion of >250 mg).
3. Increase in soft tissue plasmacytomas by 50% as measured serially by the sum of the products of the cross diameters of each measurable lesion.
4. Definite appearance of new lytic bone lesions or increase in the size of the existing bone lesions by 50%. Caution must be exercised to avoid rating progression or relapse on the basis of variation of radiologic technique alone. When progression is based on skeletal disease alone, it should be discussed with the PI before removing the patient from study.

### **OR**

5. A > 50% increase in serum or urine M-protein as defined above plus one of the following will constitute relapse or progression:
  - Hypercalcemia > 12 mg/dL without other cause.
  - Anemia (decrease in hemoglobin > 2g/dL to a level < 11g/dL in men or < 10g/dL in women). Chemotherapy or alpha2b-IFN-induced anemia or myelodysplastic syndrome with anemia do not constitute criteria for relapse of myeloma.
  - Increase in bone marrow plasma cell percentage by >50%.
  - Generalized bone pain.