CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010
DIVISION OF HEMATOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: A Phase II Trial of Allogeneic Peripheral Blood Stem Cell Transplantation From Matched Unrelated Donors in Patients with Advanced Hematologic Malignancies and Hematological Disorders

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 01089 **PROTOCOL DATE: 01/13/2020**
DATE(S)/ OF AMENDMENT(S)/REVISION(S):

COH Amendment 01	01/18/02	Version: 01
COH Amendment 02	08/06/02	Version 02
COH Amendment 03	01/15/03	Version 03
COH Amendment 04	09/12/03	Version 04
COH Amendment 05	03/10/04	Version 05
COH Amendment 06	11/18/04	Version 06
COH Amendment 07	01/16/05	Version 07
COH Amendment 08	03/25/05	Version 08
COH Amendment 09	03/16/06	Version 09
COH Amendment 10	10/14/2011	Version 10
COH Amendment 11	09/06/13	Version 11
COH Amendment 12	01/13/2020	Version 12

COH Amendment 13 at Continuation Protocol Dated 01/13/2020 (tp) Packet: 13

SITE:	Bone Marrow
STAGE (If applicable):	Advanced
MODALITY:	Allogeneic peripheral blood stem cell transplant from unrelated donor
TYPE:	Phase II
PRINCIPAL INVESTIGATOR:	Monzr Al Malki, M.D.
COLLABORATING INVESTIGATOR(S):	Stephen Forman, M.D. Joyce Niland, Ph.D.
PARTICIPATING CLINICIANS:	Ravi Bhatia, M.D., Amrita Krishnan, M.D., Ryotaro Nakamura, M.D., Leslie Popplewell, M.D., Anthony Stein, M.D. Eileen Smith, M.D., Joseph Alvarnas, M.D., Jeffrey Wong, M.D., Anna Pawlowska, M.D., Joseph Rosenthal, M.D.

CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010
DIVISION OF HEMATOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: A Phase II Trial of Allogeneic Peripheral Blood Stem Cell Transplantation From Matched Unrelated Donors in Patients with Advanced Hematologic Malignancies and Hematological Disorders

CITY OF HOPE PROTOCOL NUMBER/VERSION:	IRB # 01089 Version 12
DATE(S)/ OF AMENDMENT(S)/REVISION(S):	Amendment 1/18/02 - COH Version: 01 Amendment 8/6/02 – COH Version 02 Amendment 1/15/03 – COH Version 03 Amendment 9/12/03 – COH Version 04 Amendment 3/10/04 – COH Version 05 Amendment11/18/04 - COH Version 06 Amendment 01/6/05 – COH Version 07 Amendment 03/25/05- COH Version 08 Amendment 03/16/06- COH Version 09 Amendment 10/14/11- COH Version 10 Amendment 09/06/13- COH Version 11 Amendment 01/13/20- COH Version 12
SITE:	Bone Marrow
STAGE (If applicable):	Advanced
MODALITY:	Allogeneic peripheral blood stem cell transplant from unrelated donor
TYPE:	Phase II
PRINCIPAL INVESTIGATOR: *Designs, responsible for study conduct and data analysis*	Monzr Al Malki, M.D.
COLLABORATING INVESTIGATOR(S): *Key individual; Assists in study design and development; May be involved in analysis of data*	Stephen Forman, M.D. Joyce Niland, M.D.
PARTICIPATING CLINICIANS: *Enrolls and follows eligible patients on study; Not involved in study development or data analysis (Physician or research support staff)*	Ravi Bhatia, M.D., Amrita Krishnan, M.D., Ryotaro Nakamura, M.D., Leslie Popplewell, M.D.,
Key individual; Assists in study design and development: May be involved in analysis of data	David Snyder, M.D. Anthony Stein, M.D. Eileen Smith, M.D., Firoozeh Sahebi, M.D., Warren Chow, M.D., Joseph Alvarnas, M.D., Jeffrey Wong, M.D., Anna Pawlowska, M.D., Joseph Rosenthal, M.D.

TABLE OF CONTENTS

1.0 INTRODUCTION

Reported studies have shown that mobilized autologous peripheral blood stem cells (PBSC) can engraft patients following myeloablative therapy. The rate of engraftment following autologous stem cell transplantation (PBSCT) is reported to be more rapid with PBSC than with bone marrow. Shorter engraftment times have resulted in a shorter duration of hospitalization and a reduction in transfusion requirements.

Hesitation to use PBSC for allogeneic transplantation can be attributed to reluctance to administer cytokines to normal donors and concern that the number of lymphocytes in PBSC infusions may increase the risk of graft versus host disease (GVHD). Studies now indicate that Filgrastim (G-CSF) can be administered safely to normal volunteers, and that they can undergo apheresis with minimal side effects.(1) In addition, recent randomized studies have suggested that PBSCT do not result in a higher rate of severe GVHD in matched sibling transplants when compared to BM transplants even though up to tenfold as many T-cells may be infused with PBSC, provided that adequate GVHD prophylaxis is given.(2) Moreover, typical PBSC products contain 3-4 times more CD 34+ cells than do typical marrow products. These larger stem cell doses may be beneficial since marrow cell doses were independently associated with improved transplant outcome, especially following unrelated donor transplants.(3) Recent studies have also shown that immunologic recovery is more rapid following allogeneic PBSCT.(4) Thus the potential advantages of allogeneic PBSCT include rapid engraftment resulting in decreased transplant-related mortality, rapid immunologic recovery, and higher number of stem cells infused. This study proposes to evaluate the outcome of allogeneic PBSCT from unrelated donors for patients with hematologic disorders and hematologic malignacies.

2.0 BACKGROUND

2.1 Mobilization of Normal Donors

There is increasing experience using filgrastim (granulocyte-colony stimulating factor, G-CSF) in normal donors. Several investigators have treated many healthy volunteers with G-CSF for five to six days. The most common adverse effect experienced by the donors was bone pain. Other symptoms include nausea, vomiting, and influenza-like symptoms, including headache, fatigue, myalgia, and night sweats.(5) During apheresis, donors may experience bone pain, which was relieved by analgesics, and a decrease in platelet counts and hematocrit. The decrease in platelet count and hematocrit appears to be due in part to numbers of platelets and red cells contaminating in the stem cell products, and do not fall below a clinically significant range. None of the donors required blood product transfusions. There have been no long-term side effects with follow-up of greater than one year.

Mobilized PBSC from healthy allogeneic transplant donors are now being investigated as an alternative to bone marrow stem cells. Lane et al demonstrated that it is feasible to collect sufficient PBSC from normal donors for allogeneic transplantation by mobilizing with G-CSF, or GM-CSF, or a combination of both with minimal risk to the donor.(6) Other published regimens for mobilization and collection of normal donor PBSC typically include a dose of ≥ 10 μg/kg/day of rhG-CSF with apheresis initiated after the fourth dose of G-CSF (day 5).(7) Using this regimen, peak blood levels of CD34+ cells on day 5 have been reported to be in the range of 33-113/μL, depending on the center, with a wide range of CD34 cells collected. Schmitz et al report collection of 3-8 x 10^6 CD34 cells/kg recipient weight with 1-3 standard volume apheresis procedures. Urbano-Ispizua et al reported a median collection of 6 x 10^6 CD34 cells/kg recipient weight with 1-2 standard volume aphereses.(8) Brown et al report a mean of 10^7 CD34cells/kg recipient weight with most (91%) donors requiring only a single large volume apheresis.(9) Using a higher dose of G-CSF for mobilization (16 μg/kg/day), Bensinger et al report collecting a median of 13 x 10^6 CD34 cells/kg recipient weight in two standard volume apheresis procedures.(10) While the optimal mobilization and apheresis schedule is not clear, it is clear that sufficient numbers of PBSC can be routinely collected.

Potential advantages of the use of mobilized PBSC in allogeneic transplantation include elimination of the need for hospitalization, general anesthesia and blood transfusions for the donor and potential for accelerated neutrophil and platelet engraftment for the recipient (compared to BM). Potential disadvantages of allogeneic PBSC transplantation include the theoretical increased risk of GVHD due to the presence of large numbers of T lymphocytes in the harvest. Recent reports have suggested that although the incidence and severity of acute GVHD are not increased, the incidence of chronic GVHD may be significantly higher in recipients of PBSC.(11)

Currently, the National Marrow Donor Program has announced the availability of G-CSF stimulated PBSCs for hematopoietic transplants from unrelated donors, as an alternative to bone marrow. NMDP donors who elect to donate PBSC will participate in NMDP protocol entitled " Filgrastim-Mobilised Peripheral Blood Stem Cells for Primary Allogeneic Transplantation with Unrelated Donors"

2.2 Graft versus Host Disease

In transplants from matched related donors, early results suggest that recipients of unselected PBSC from matched sibling donors experience acute GVHD at rates similar to recipients of BM from matched sibling

donors despite the large difference in T cell content, provided that they receive adequate GVHD prophylaxis (two drug therapy). Even with adequate immunosuppressive therapy, approximately 35% of recipients of bone marrow from matched sibling donors will develop moderate to severe acute GVHD and at least 30% will develop manifestations of chronic graft versus host disease.

Adequate GVHD prophylaxis typically consists of cyclosporine plus methotrexate or prednisone. Clinical studies have demonstrated a significant reduction in acute GVHD and associated improved survival using a combination of methotrexate and cyclosporine compared to cyclosporine alone in leukemia patients, however, the addition of methotrexate is also associated with potential engraftment delays due to its myelosuppressive effects.(12)

The use of FK-506 (Prograf) for the prevention of AGVHD has been reported since 1995.(13) In a randomized trial comparing CSA and FK-506 with MTX for AGVHD prophylaxis in allogeneic matched sibling transplant patients, FK-506 was found to be more effective in AGVHD prevention but patients with advanced disease had a higher incidence of regimen related toxicity and a lower survival.(14) A separate randomized trial between FK-506 and CSA with MTX for AGVHD prophylaxis in unrelated donor transplant patients showed an incidence of grade II-IV AGVHD of 56% for FK-506 and 74% for CSA with no difference in long term survival.(15) Another Phase II trial with FK-506 and MTX for AGVHD prophylaxis in unrelated donor transplants showed an incidence of grade II-IV AGVHD of 34% compared to 43% incidence of grade II-IV acute GVHD reported in 1423 unrelated donor transplant patients given CSA/MTX, T Depletion or other prophylaxis. FK-506 has also been shown to work synergystically with MMF to treat chronic GVHD in 26 patients who had progressed after standard therapy with CSA and prednisone with an objective response rate of 46%.

Mycophenolate mofetil (MMF) is the 2-(4-morpholino) ethyl ester of mycophenolic acid (MPA). MMF is rapidly absorbed following oral administration and hydrolyzed to the active MPA which blocks purine synthesis in the T and B lymphocytes. Preclinical studies in allogeneic transplant models demonstrate activity in preventing graft rejection and GVHD. Preliminary evidence after transplantation of unrelated DLA-mismatched marrow in dogs is that MMF synergizes with CSA to prevent GVHD and improve survival; in this unrelated transplant model the combination of CSA/MMF was more powerful in preventing GVHD and rejection than CSA/MTX.(16) Additional clinical studies using oral CSA/MMF for acute GVHD prophylaxis in the non-myeloablative setting have further demonstrated that this combinationis very effective in

preventing AGVHD and leads to less mucositis than expected with MTX.(17)

With pre-clinical studies showing the drug is more effective in AGVHD prevention and clinical data showing there is less mucositis compared to MTX the drug has become a prime candidate for clinical studies in AGVHD prophylaxis. It's use in AGVHD prevention in the myeloablative allogeneic transplant setting had not been studied until recently due to the lack of availability of an intravenous (IV) formulation which would allow use during the post high dose chemotherapy and radiation phase of mucositis. IV MMF is now available. We have currently approved a multi-institutional protocol (IRB#99106) to study the use of IV MMF in combination with CSA for AGVHD prophylaxis in allogeneic sibling BMT patients.

2.2.1 Sirolimus

Sirolimus (Rapamune®, Wyeth-Ayerst) is a commercially available immunosuppressant licensed for use in solid organ transplantation. Sirolimus is a macrocyclic lactone similar in structure to tacrolimus and cyclosporine but with different mechanism of action from the calcineurin inhibitors. Sirolimus was shown to be synergistic with cyclosporine and tacrolimus in animal models and has a distinct toxicity profile, allowing their use in combination. In the solid organ transplantation setting, sirolimus has rapidly become the standard of care, allowing for reduced doses of calcineurin inhibitors and as a consequence, less renal toxicity. In addition, the use of sirolimus has lead to improved allograft survival without an increase in the rate of serious infections.

11/18/04

Sirolimus is a macrocyclic lactone immunosuppressant derived from *Streptomyces hygroscopicus* that is similar in structure to tacrolimus. Sirolimus has excellent antirejection activity in organ transplantation (11) and combination therapy with cyclosporine or tacrolimus appears to be extremely effective in human organ allografting (12-14) and mouse models of hematopoietic stem cell transplantation (HSCT) (15,16). There is also a study demonstrating the activity of sirolimus in steroid-resistant graft-versus-host disease (GVHD) (17).

Sirolimus binds to the same family of intracellular FK-506 (tacrolimus) -binding proteins (FKBP12 and others) at a site distinct from tacrolimus. Sirolimus-FKBP complexes inhibit the mammalian target of rapamycin (mTOR), a kinase that regulates cell cycle entry in response to interleukin 2 (IL-2) signaling and other cellular functions. Cell cycle entry in the presence of sirolimus induces T cell apoptosis, and deprivation of IL-2 signaling renders antigen-activated T cells unresponsive. Both mechanisms are thought to contribute to T cell immunologic tolerance.

mTOR suppresses cytokine-driven T cell proliferation by inhibiting: 1) transcription of a family of mRNA's needed for cell cycle progression; 2) IL-2 induced transcription of PCNA which is necessary for DNA replication; 3) CD28-mediated upregulation of IL-2 transcription; 4) kinase activity of cdk4/cyclin B and cdk2/cyclin E complexes thus preventing cell cycle progression. Thus, G1→ S progression is blocked. Sirolimus may also inhibit dendritic cell function (18, 19, 20, 21, 22, 23).

While both tacrolimus and sirolimus bind to FKBP12, there appear to be adequate binding sites for both molecules. Therefore, contrary to expectations, the drugs are not competitive, and, in fact, they appear to be synergistic (24, 25, 26). Sirolimus also is free of nephrotoxicity and neurotoxicity, making combination therapy appealing. Sirolimus affects lymphocyte activation at a later stage than either cyclosporine or tacrolimus, and activation stimuli that resist inhibition to the latter agents have been shown to be sensitive to sirolimus. Despite the activity observed in organ transplantation, its application to HSCT was delayed by concerns about bioavailability of an oral agent, as well as neutropenia and thrombocytopenia, which may complicate its use.

2.3 Experience with Sirolimus Prophylaxis in Unrelated or Mismatched-Related Transplants

Investigators from Boston performed an open label phase 1/2 trial in 39 patients receiving a mismatched related (n=5) or unrelated donor (n=34) transplant (36). Patients were conditioned with cyclophosphamide and TBI (14 Gy). GVHD prophylaxis consisted of tacrolimus/sirolimus and MTX 5 mg/m2 on days 1, 3, 6 and 11 post-transplant. The median age was 42 (19-62). Diagnoses were CML (12), ALL (9), AML (10), MDS (7) and NHL (2). Despite its oral formulation, sirolimus blood levels of 3-12 ng/mL was maintained in most patients. All patients attained durable engraftment, with median times to neutrophil and platelet engraftment of 18 days and 29 days, respectively. Grade 0-1 acute GVHD occurred in 75% of patients. Grades 2, 3, and 4 acute GVHD occurred in 13%, 8%, and 5%, respectively (total grades 2-4 GVHD, 26%). Mortality from RRT included hepatic VOD (3), IP (2), infection (3), hemorrhage (1), and multiorgan failure (1). Sirolimus-related toxicities were hyperlipidemia, which was well controlled with lipid lowering agents, and 1 case of HUS which resolved with reduction of sirolimus and tacrolimus doses. Relapse was observed in 10 patients. 100-day survival was 80%. The 1-year RFS and OS were 52% and 45%, respectively.

2.4 PBSC Transplantation: Related Donors

In a retrospective study reported by Bensinger et al, 37 patients transplanted with unselected PBSC from HLA-matched siblings and the outcomes were compared to an historical group of bone marrow

transplants.(18) Donor mobilization was with G-CSF, 16 μg/kg/d for five days, with PBSC collections on days 4 and 5. Average age of the PBSC group was 38 (range 20-52 years). The diagnoses included AML (41%), NHL (24%), ALL (14%), Hodgkin's disease (11%), CML (5%) and multiple myeloma (5%). The majority of patients were relapsed (89%), 5% were in >second remission, and 5% were CML patients in blast crisis. GVHD prophylaxis was either CSA plus MTX in 19/37 (51%) or CSA plus prednisone in 18/37 (49%). Grade II-IV acute GVHD occurred in 37% of the recipients.

The median time to neutrophil engraftment (ANC >500/μL) was 14 days in the PBSC group compared to 16 days in the historical bone marrow controls (p=0.0063), and to platelet engraftment (first of 7 consecutive days with >20,000/μL) was 11 and 15 days respectively for the PBSC and bone marrow groups. These results suggest that allogeneic PBSC approach was associated with faster engraftment, fewer transfusions and no greater incidence of acute or chronic GVHD.

Several Phase II studies of PBSC transplant from matched sibling donors have been reported by several investigators. Most patients received PBSC products containing $\geq 5 \times 10^6$ CD34+ cells/kg. Engraftment was rapid in all patients with no late graft failure observed. All patients received various GVHD prophylaxis regimens. The incidence of grade II-IV acute GVHD were similar to historical BMT data for HLA-identical pairs. However, the risks of chronic GVHD were higher in some studies.

2.5 Randomized Studies Comparing Allogeneic BMT vs. PBSCT in Matched Siblings

Two prospective, randomized clinical trials with relatively small numbers of patients comparing PBSC and BM have been reported.(19) Time to platelet recovery was shorter in the PBSC group in both studies, and neutrophil engraftment was shorter in one study. Acute GVHD incidence, DFS, and LFS did not differ between the groups in either study, but a trend towards a higher incidence of chronic GVHD was seen in both studies, a finding suggested by a large retrospective study reported by the International Bone Marrow Transplant Registry (IBMTR).(20)

A large prospective randomized study of PBSC or BM for patients undergoing allogeneic stem cell transplant was recently reported by Bensinger et al. on behalf of investigators from the FHCRC, COHNMC and Stanford University.(2) PBSC transplantation was associated with faster engraftment without any difference observed in acute or chronic GVHD at a median follow-up of 20 months. Although survival differences were not seen in patients with good risk disease, PBSC transplantation was associated with lower transplant-related mortality and

relapse in patients with advanced disease. Similar results from two additional large studies from Canada and Europe have also been reported.

2.6 BM Transplantation: Unrelated Donors

Approximately 70% of patients with hematologic malignancies and other hematologic disorders for whom allogeneic stem cell transplantation is potentially curative, lack matched sibling donors. For these patients, other options for stem cell sources for transplantation currently include partially matched relatives, matched unrelated donors (URD) and cord blood.

The National Marrow Donor Program was established in 1988 to provide bone marrow from unrelated donors to patients lacking suitable family donors. As of December 1998, the NMDP had facilitated more than 7,860 unrelated donor transplants. Essentially all of these represent transplants of bone marrow collected from donors during general or regional anesthesia. Over the last few years NMDP has established procedures to allow the collection of PBSC as an alternative to bone marrow in the second donation setting. As the experience with allogeneic PBSC has grown, PBSC is now being used as an alternative to bone marrow for first donation.

Data from 1384 patients receiving URD BMT between November 1991 and August 1995 facilitated by the NMDP have been recently analyzed.(21) These data summarize the experience using bone marrow in these transplant pairs and should serve as the baseline for examining the initial data from unrelated donor PBSC transplant. Sixty-three (63%) of the pairs in the NMDP summary (n = 869) were matched at HLA-A, B (serological) and DRB1 (molecular). HLA-C typing was not available for the majority of pairs. T-cell depletion was performed for the prevention of GVHD in 30% of the cases.

Neutrophil engraftment (defined as the first of three consecutive days on which ANC > 500/μL) occurred at a median time of 20 days after marrow transplantation (all patients). Although neutrophil engraftment was more rapid in patients who received T-depleted marrow (median 17 days), and in patients not given methotrexate, both of these groups were at greater risk for graft failure. Neutrophil engraftment by day 42 occurred in 92% of patients who received either T-depleted marrows or no methotrexate compared to 96% of patients receivingT-replete marrows and 95% of patients who received methotrexate. Delay of engraftment beyond 42 days was associated with reduced survival. Primary graft failure was reported to occur in 5% of patients, and secondary graft failure in 14%. Recipients of T-depleted grafts were not at higher risk for secondary graft failure.

Platelet engraftment (defined as >50,000/μL without transfusion for seven days) occurred at a median time of 82 days after marrow transplant, and was achieved by 54% of recipients by day 100. Favorable risk factors included higher cell dose, HLA-A, B and DRB1 matching, CMV seronegative recipients and female recipients. Platelet recovery was significantly slower in patients with Grades III-IV GVHD. In this series of patients, platelet engraftment was an important predictor of survival, and appears to be a valuable clinical indicator of likely outcome.

In a separate analysis of 1423 consecutive CML patients transplanted with unrelated donor bone marrow reported rates of severe acute (Grades III-IV) and extensive chronic GHVD were 33% and 60%, respectively.(22) In this series, primary graft failure occurred in 10.3% of patients and secondary graft failure in 6.9%.

2.7 PBSC for Unrelated Donor Transplantation

Reports of PBPC transplants from unrelated donors have recently been published. Ringden and colleagues reported successful and faster engraftment of platelets and neutrophils without significant increase in acute GVHD in patients receiving PBSC from unrelated donors without differences in acute GVHD or survival.(23) A case control study of 90 patients receiving transplants from unrelated donors was conducted, with 45 patients receiving PBPC and 45 patients receiving BM.(24) A short time to platelet count recovery was seen in the PBPC group. Grade II - IV acute GVHD was 20% in the BM controls and 30% in the PBPC group. The probability of chronic GVHD was 85% in the BM groups, 59% in the PBPC group. One-year transplant related mortality (TRM), and overall survival differences were not significant.

2.8 Clinical Effect of HLA Matching in Unrelated Donor Marrow Transplantation

Important advances have been made in understanding how the HLA system relates to the success of hematopoietic sell transplantation. It has been known for sometime that HLA disparity increases both the risks of GHVD and graft rejection.(25) Recent studies using molecular techniques have suggested that graft rejection is primarily attributable to disparity of class I alleles, while GVHD has been attributed to disparity of class II alleles. In retrospective study reported by Petersdorf et al, molecular mismatch at HLA-C was found to be an independent risk factor for graft failure.(26)There was also a trend toward an increased risk of rejection with single HLA-A, or –B mismatches. The incidence of rejection increased with multiple class I allele mismatches.

GVHD was also related to HLA disparity. (27)Early studies with HLA mismatched related donors demonstrated an increasing incidence and severity of GVHD with increasing numbers of mismatches. In related donor setting, a single antigen mismatch at HLA-DR was associated with higher risk of GVHD than single antigen mismatch at HLA-A, or –B.(28)

In unrelated donor setting, several of studies have shown that there was an increased risk of GVHD in serologically matched unrelated donor recipients compared to matched related donor recipients.(29) In a study performed at the Fred Hutchinson Cancer Research Center (FHCRC), using ssop typing, increased risks of GVHD and death were found in HLA-DRB1 mismatched patients.(30) A subsequent study also found that molecular mismatching at DQB1 conferred a significantly increased risk of GVHD that was further increased with a combined DRB1 and DQB1 mismatch. A single class I allele mismatch did not increase the risk of GVHD. However, multiple Class I and Class II mismatches increased the risk of GVHD.(31)Contrary to the findings at the FHCRC, a recent Japanese study found that class I disparity at HLA-A and –C alleles contributed significantly to the risk of GVHD and death.(32) Class II disparity, in that study, did not contribute to GVHD or death. The differences in results are, as yet, unexplained.

2.9 Rationale for Allogeneic PBSCT from Unrelated Donors

The recent analysis of 1423 consecutive CML patients transplanted with unrelated donor bone marrow facilitated by the NMDP indicates that overall survival is similar in recipients of related and unrelated marrow grafts. Further, in good risk cohorts of patients, the 3-year disease free survival was similar in both groups. Interest in the use of mobilized PBSC from unrelated donors has increased as experience with the use of mobilized PBSC from related donors has increased. Based on this experience, there appear to be several potential advantages of the use of mobilized PBSC in the unrelated setting, including more rapid engraftment, decreased transplant-related morbidity and mortality, and the ability to provide large doses of stem cells.

Potential disadvantages may be related to the increased number of T-cells infused with mobilized PBSC and the potential for increased GVHD, acute or chronic. This increased risk of chronic GVHD may contribute to long term complications, increased costs and decreased quality of life in these patients.

3.0 OBJECTIVES

This is a Phase II study designed to evaluate the hematopoietic recovery, the incidence of acute and chronic GVHD and survival outcome of patients with

advanced hematologic malignancies or hematological disorders who are undergoing PBSC transplants from matched unrelated donors. The criteria for HLA matching in this protocol, in general, will be similar to those used at the City of Hope for conventional unrelated BMT. Donors and recipients should be matched for HLA –A, -B, and -DRB1 at least to the level of high resolution molecular typing. If possible, matching for HLA -C, and -DQB1 alleles will be preferred, but not mandatory.

3.1 Primary Objectives

The primary objectives of the study are:

1. To evaluate hematopoietic recovery, using neutrophil and platelet engraftment as the primary criterion, following unrelated donor PBSC transplant.

2. To evaluate the incidence of acute and chronic GVHD in recipients of matched unrelated donor PBSC transplant.

3.2 Secondary Objectives

1. To evaluate the impact of HLA class I and class II alleles matching on the incidence of GVHD and on the survival outcome following PBSCT from unrelated donors.

2. To evaluate the overall survival, disease-free survival and relapse following unrelated donor PBSCT. Using both myeloablative and non-myeloablative conditioning regimens.

4.0 STUDY DESIGN

This is a phase II trial of allogeneic matched unrelated PBSC transplants for patients with Hematologic malignancies or disorders who do not have matched sibling donors, or are not a candidates for autologous stem cell transplant.

Patients must have a matched unrelated donor who meets donor evaluation criteria and who has agreed to the mobilization and apheresis procedures according to National Marrow Donor Program Standard. Several preparative regimens will be used depending on their diagnosis, their ages, their disease status at transplant and whether they had a prior transplant. All patients will receive unrelated donor PBSC transplant. Three different GVHD prophylaxis will be used either the combination of FK 506 (prograf) and methotrexate, or the combination of cyclosporine, mycophenolate mofetil (MMF) and mini-methotrexate, or the combination of FK 506, sirolimus and mini-methotrexate.

8/6/02

1/18/02, 1/15/03 11/18/04

All observed toxicities and side effects will be recorded, and the severity and degree of GHVD will be assessed. Toxicities and side effects of particular interest include engraftment delays, renal toxicity, hepatic toxicity, and graft rejection or failure. Durability of engraftment will be monitored by following hematopoietic parameters for a minimum of 6 months following transplantation. Acute and chronic GVHD will be assessed and documented using City of Hope criteria. Patients will be seen twice a week during the first 100 days then weekly until 6 months post transplant, and at 12, 18, and 24 months after the transplant. Patients will be followed at least for two years for disease-free and overall survival and then indefinitely.

Matched unrelated donors will be identified through standard donor search procedures, and will be screened, mobilized, and apheresed according to usual NMDP standards.

5.0 STUDY POPULATION

Male or female patients who meet eligibility requirements and for whom an HLA-identical unrelated donor has been identified.

6.0 PATIENT SELECTION

6.1 Inclusion Criteria : The following conditions must be met before a patient may be enrolled in the study.

6.1.1 There is no upper age limits for this protocol. Patients age > 55 years with hematologic diseases treatable for allogeneic stem cell transplant who are not eligible for IRB # 99190 will be enrolled on this protocol.

6.1.2 Disease Criteria:

Patients with ALL in first relapse or beyond, or high risk ALL defined as:

- hypoploidy (≤ 44 chromosomes)
- pseudodiploidy with translocations or molecular evidence of t(9;22), 11q23, or t(8;14), excluding B-ALL
- elevated WBC at presentation (>20,000/mm^3 >18 yrs; >200,000/mm^3 12-18 yrs);

OR

Patients with AML in 1CR, or failed to achieve remission (IF), or first relapse or beyond; 11/18/04

OR

Patients with CML in first or second chronic phase or accelerated phase, or blast crisis, (Blast crisis is defined as > 30% promyelocytes plus blasts in the bone marrow.)

OR

Patients with myelodysplastic syndrome (MDS), including RAEB, CMML, and RAEB-T. Patients with secondary AML (> 30% blast in the marrow aspirate) should receive induction chemotherapy to obtain a remission if possible before transplant. Patients with myeloproliferative disorders (MPS)/myelofibrosis can be considered on a case by case basis and transplant must be approved by review committees and the principal investigator.

OR

Patients with refractory NHL, CLL, HD and Multiple Myeloma who have received and failed frontline therapy, high-dose therapy and autologous stem cell transplant or salvage therapy. 9/12/03

OR

Patients with severe aplastic anemia, or PNH, or any other hematologic disorder requiring transplantation 11/18/04

6.1.3 Patients with consenting unrelated volunteer donor that meets donor selection criteria.

6.1.4 Patients with adequate organ function as measured by:

Cardiac: Left ventricular ejection fraction at rest must be ≥ 45%.

Hepatic: SGOT within 2x normal range and total bilirubin <1.5x normal range unless liver function abnormalities are related to underlying disease.

Renal: Serum creatinine within 1.5x normal range or creatinine clearance ≥ 60 ml/min.

Pulmonary: DLCO (diffusion capacity) ≥ 40% of predicted (corrected for hemoglobin).

6.1.5 Written informed consent conforming to institutional guidelines obtained from patient or parent (if a minor).

6.1.6 Life expectancy > 8 weeks and absence of co-existing medical problems which would significantly increase the risk of the transplant procedure in the judgment of the principal investigator.

6.2 Exclusion Criteria : Any one of the following conditions eliminates a patient from participating in this protocol.

6.2.1 Availability of a 6/6 matched related donor.

6.2.2 Uncontrolled CNS involvement with disease.

6.2.3 Female who are pregnant

6.2.4 Karnofsky performance status < 50%.

6.2.5 Seropositivity for HIV.

6.3 Donor Selection

6.3.1 Donors must meet criteria set forth by the NMDP for donors of Filgrastim-mobilized peripheral blood stem cells for primary allogeneic transplantation. Please refer to the NMDP protocol entitled "Filgrastim-Mobilized Peripheral Blood Stem Cells for Primary Allogeneic Transplantation with Unrelated Donors" (City of Hope IRB #99064).

6.3.2 HLA-phenotypically identical with the patient for HLA-A, and –B and identical for DRB1 alleles. Matching assessed by DNA typing for both Class I and Class II antigens. Allele mismatch for HLA class I (i.e. B 2701 vs. B 2702) is acceptable if no alternative donors. Allele mismatch for class II (i.e. DRB1 0401 vs. 0402), or minor mismatch for class I Cross Reactive Group (CREG, i.e. A 2 vs. A 28) is acceptable in patient age ≤ 35 years old requiring urgent transplant.

6.3.3 Written informed consent from donor.

6.4 Registration

At the time informed consent is obtained, qualified patients will be assigned a COH study number.

7.0 MATERIALS AND METHODS

7.1 Mobilization and Leukapheresis (PBSC Collections)

7.1.1 G-CSF (Neupogen®)

See Appendix 14.2 for pharmaceutical information.

7.1.2 Equipment

Apheresis will be performed using a peripheral or central venous access at donor center's discretion.

7.2 Cytoreductive Therapy

See appendix for pharmaceutical information on drugs used in this study.

8.0 TREATMENT PLAN

8.1 Donor Mobilization

PBSC donors must meet the criteria set forth by the NMDP for donors of Filgrastim-mobilized peripheral blood stem cells for primary allogeneic transplantation. Please refer to the NMDP protocol entitled "Filgrastim-Mobilized Peripheral Blood Stem Cells for Primary Allogeneic Transplantation with Unrelated Donors"

8.1.1 Donor search, donor selection, donor work-up and donor informed consent are per NMDP protocol.

8.1.2 Eligible donors will begin mobilization with G-CSF at approximately 10 μg/kg/day subcutaneously (rounded off to full vials) per NMDP protocol.

8.1.3 Starting on Day 5 of mobilization, two large volume apheresis procedures will be performed. The first apheresis will be on Day 5 and the second on Day 6. PBSC will be collected using the Fenwal CS-3000, COBE Spectra or comparable automated cell separator according to established procedures. Typically 15-20 liters of blood will be processed during each apheresis collection. Both apheresis product will be shipped via courier after completion of collection to the Transplant Center.

8.1.4 The goal is to collect and infuse $\geq 5.0 \times 10^6$ CD34 + cells/kg recipient weight, if possible.

8.1.5 G-CSF administration to the donor may be modified at any time during the mobilization procedure if the WBC exceeds 75,000/μL. The following table summarizes appropriate schedules for donor mobilization that may be used.

	M	Tu	W	Th	F	Sa	Su	M	Tu	W	Th	F
I	SF	RF		F1	F2	F3	F4	F5 A1	 A2			
II		SF	RF		F1	F2	F3	F4	F5 A1	 A2		
III			SF	RF		F1	F2	F3	F4	F5 A1	 A2	
IV				SF	RF		F1	F2	F3	F4	Fg5 A1	 A2

SF: Ship Filgrastim, RF: Receive Filgrastim, F*x*: Administer day *x* of Filgrastim, A*x*: Perform day *x* of apheresis.

8.2 **Conditioning regimens**: Several preparative regimens will be used. The choice of regimen will be depending upon patients' diagnoses, age, disease status, prior radiation therapy and prior autologous stem cell transplant (ASCT). Reduced-Intensity myeloablative regimens will be recommended in: older patients, prior ASCT, and those with underlying medical problems which place them at increased the risk of transplant-related morbidity or mortality.

8/6/02

Regimen I: TBI and Cyclophosphamide

Day -8	Admission	
Day -7	TBI	120 cGyx3
Day -6	TBI	120 cGyx3
Day -5	TBI	120 cGyx3
Day -4	TBI	120 cGyx2
Day -3	Cyclophosphamide	60 mg/kg (IBW)
Day -2	Cyclophosphamide	60 mg/kg (IBW)
Day 0	Infusion of PBSC.	

For patients who weigh ≤ 120% of ideal body weight, the dose of Cy will be based on the patient's ideal body weight. For patients who weigh > 120% of ideal body weight, the dose of Cy will be based on the adjusted ideal body weight (AIBW), calculated as follows:

AIBW = Ideal Body Wt. + [(0.25) x (Actual Body Wt - Ideal Body Wt)]

To prevent hemorrhagic cystitis, Mesna will be administered intravenously before cyclophosphamide at a dose equal to 40% of cyclophosphamide dose over 15 minutes, and then will be repeated every 3 hours for a total of 48 hours.

To accommodate stem cell arrival date and total body radiation schedule, cyclophosphamide may be given before TBI as follows: 1/18/02

Cyclophosphamide and TBI

Day – 7	Cyclophosphamide 60 mg/kg IBW
Day – 6	Cyclophosphamide 60 mg/kg IBW
Day – 5	Rest day
Day – 4	TBI 120 cGyx3
Day – 3	TBI 120 cGyx3
Day – 2	TBI 120 cGyx3
Day – 1	TBI 120 cGyx2
Day – 0	Infusion of PBSC

3/25/05

[If patients receive Tacrolimus/Sirolimus/MTX for GVHD prophylaxis the conditioning regimen (Regimen I) will be started two days earlier so sirolimus will not be given the same day with chemotherapy to prevent interaction between chemotherapy and sirolimus. There will be no change in the conditioning schedule if Cyclophosphamide is given prior to TBI as allowed to accommodate stem cell arrival and TBI schedule.]

Regimen II: Busulfan and Cyclophosphamide

Day -12	start dilantin 300 mg p.o. t.i.d. x 1 day, then 300mg p.o.daily for 10 days.
Day-8	Admission, dilantin blood levels will be checked and dose adjusted as needed to meet therapeutic range. Further adjustments if clinically indicated. Busulfan test dose administered at 6 a.m. as a single dose. The I/V dose is calculated as follows:

1. Body surface area (BSA) is calculated by the equation:

$$BSA = \sqrt{\frac{(\text{Actual body weight(kg)} \times \text{height (cm)})}{3600}}$$

2. 0.8 mg/kg adjusted BW) I/V Busulfan will be given over 2 hours. Blood levels will be obtained with the first dose as per Appendix G and shipped via overnight mail to Quest Diagnostics. Inc. 8/6/02

Day –7

Busulfan AUC will be calculated by standard methods available in the City of Hope Analytical Pharmacology Core Facility (APCF) or those standards used at that site. The resulting AUC will be used to determine the dose required to achieve target AUC of 1200 µM* min (range 800-1400) according to the following formula: 8/6/02

$$\text{Adj dose} = \text{Current dose} \times \left(\frac{800 \mu M \times \text{min}}{\text{test dose AUC}} \right)$$

The maximum dose given will not exceed 32 mg/m^2. There is no limit on dose reduction Busulfan dose will only be adjusted for AUC's <800. 8/6/02

Day -7	Busulfan 0.8 mg/kg q 6 hrs starting at noon
Day-6	Busulfan 0.8 mg/kg q 6 hrs
Day-5	Busulfan 0.8 mg/kg q 6 hrs
Day –4	Busulfan 0.8 mg/kg q 6 hrs; total 16 doses
Day –3	Cyclophosphamide 60 mg/kg IBW
Day –2	Cyclophosphamide 60 mg/kg IBW
Day 0	PBSC infusion

8/6/02

Busulfan will be dose calculated based on Adjusted Body Weight. 8/6/02
To prevent hemorrhagic cystitis, Mesna will be administered intravenously before cyclophosphamide at a dose equal to 40% of cyclophosphamide dose over 15 minutes, and then will be repeated every 3 hours for a total of 48 hours

[If patients receive Tacrolimus/Sirolimus/MTX for GVHD prophylaxis the conditioning regimen (Regimen II) will be started two days earlier so sirolimus will not be given the same day with chemotherapy to prevent interaction between chemotherapy and sirolimus.] 3/25/05

Regimen III: TBI and VP-16 11/18/04

Day-7	TBI 120 cGyx3
Day-6	TBI 120 cGyx3
Day-5	TBI 120 cGyx3
Day-4	TBI 120 cGyx2
Day-3	Etoposide 60 mg/kg (adjusted BW)
Day-0	PBSC Infusion

8/6/02

[If patients receive Tacrolimus/Sirolimus/MTX for GVHD prophylaxis the conditioning regimen (Regimen III) will be started one day earlier so sirolimus will not be given the same day with chemotherapy to prevent interaction between chemotherapy and sirolimus.] 3/25/05

Reduced-Intensity Myeloablative Regimen

Regimen IV: Fludarabine and Melphalan

Day-7	Fludarabine 25 mg/m^2 IV
Day-6	Fludarabine 25 mg/m^2 IV
Day-5	Fludarabine 25 mg/m^2 IV
Day-4	Fludarabine 25 mg/m^2 IV
Day-3	Fludarabine 25 mg/m^2 IV
Day-2	Melphalan 140 mg/m^2 IV
Day 0	PBSC infusion

Both Fludarabine and melphalan doses will calculated based on actual body weight.

[If patients receive Tacrolimus/Sirolimus/MTX for GVHD prophylaxis the conditioning regimen (Regimen IV) will be started two days earlier so sirolimus will not be given the same day with chemotherapy to prevent interaction between chemotherapy and sirolimus.] 3/25/05

Regimen V: Fludarabine and TBI

Day-4	Fludarabine 30 mg/m^2 IV
Day-3	Fludarabine 30 mg/m^2 IV
Day-2	Fludarabine 30 mg/m^2 IV
Day 0	TBI 200 cGy to be administered between 10.00 a.m.and 1.00 pm PBSC infusion

[For Regimen V, no change will be made to the fludarabine and TBI schedule if patients receive Tacrolimus/Sirolimus/MTX for GVHD prophylaxis.] 3/25/05

Regimen VI: Busulfan and Fludarabine	
Day-8	Begin Dilantin
Day-5	Busulfan 0.8 mg/kg IV over 3 hrs Fludarabine 30 mg/m^2 IV
Day-4	Busulfan 0.8 mg/kg IV over 3 hrs Fludarabine 30 mg/m^2 IV
Day-3	Busulfan 0.8 mg/kg IV over 3 hrs Fludarabine 30 mg/m^2 IV
Day-2	Busulfan 0.8 mg/kg IV over 3 hrs Fludarabine 30 mg/m^2 IV
Day-0	PBSC infusion

1/18/02

Calculation of Ideal Body weight

1. Male IBW= 50 kg + 2.3 kg (height [in]-60)
2. Female IBW = 45.5 kg+ 2.3kg (height [in]-60)

[If patients receive Tacrolimus/Sirolimus/MTX for GVHD prophylaxis the conditioning regimen (Regimen VI) will be started two days earlier so sirolimus will not be given the same day with chemotherapy to prevent interaction between chemotherapy and sirolimus.]

3/25/05

8.3 **GVHD Prophylaxis**

Three different GVHD prophylaxis will be allowed including:

Prograf and methotrexate.
OR
Cyclosporine, MMF and mini-methotrexate.
OR
Prograf, sirolimus and mini-methotrexate

1/18//02, 8/6/02, 1/15/03, 11/18/04

All patients should have cyclosporine and prograf levels monitored to insure a therapeutic range is achieved. (For dose reduction guidelines, see Appendix B)

8.3.1 ***GVHD PROPHYLAXIS REGIMEN A: PROGRAF+MTX***

Day –1 start FK506 .03 mg/kg/day IV until day +21 then switch to po (see below)

Methotrexate
Day +1 15 mg/m^2 IV starting 24 hours after the PBSC infusion is complete

Day +3 10 mg/m^2 IV
Day +6 10 mg/m^2 IV
Day +11 10 mg/m^2 IV

Leucovorin rescue may be used per discretion of primary Hematologist. Guidelines for MTX dose adjustment in patients with renal dysfunction, mucositis, or peripheral capillary leak are outlined in Appendix B. In such patients, MTX levels may be checked beginning 24 hours after MTX administration and if the MTX level is high, leucovorin may be administered according to institutional standards/guidelines

FK506: All patients will receive FK-506 .03 mg/kg/day IV from day –1 until day +21 , then switch to 0.1 mg/kg/day orally. Dosing should be based on adjusted ideal body weight. FK-506 whole blood levels will be monitored twice a week and adjusted to maintain therapeutic levels of 10-20 ng/ml. If GVHD is absent, FK-506 will be then tapered by 5%/week until a dose of 0.02 mg/kg/day is reached. This dose will be maintained until day 180. Further decisions on tapering FK-506 will then be made by the primary physician.

8.3.2 GVHD PROPHYLAXIS REGIMEN B: Cyclosporine, MMF and mini-Methotrexate

1/15/03

Cyclosporine

Day -1 through +21	3 mg/kg/day by continuous infusion or 1.5 mg/kg i.v. q 12 hours
Day +21 through +180	Switch to oral dose at 5 mg/kg or up to 6.25 mg/kg po BID to maintain plasma cyclosporine trough levels as measured by fluorescence polarization immunoassay (TDX) of 200-600 ng/mL (170-500 by HPLC; whole blood levels by TDX of 900-1500 ng/mL).

1/18/02

The scheduling of cyclosporine administration should be such that a full 24 hour dose has been given before the PBSC are infused. Oral dosing is not allowed prior to day 14.

Day 0 start MMF at 15 mg/kg IV BID
until day +27. May be switched to oral after day +14

11/18/04

MMF: IV will begin day 0 a minimum of 2 hours after the end of the stem cell infusion. MMF will be administered at 15 mg/kg/twice a day from day 0 until day 27. The intravenous formulation will be administered until at least day 14 after transplant. MMF will be administered as a 2 hour IV infusion. MMF will be infused with 5% dextrose. When patients have

11/18/04

recovered from radiation and chemotherapy induced gastroenteritis and are capable of taking the oral MMF, they will be converted at an oral to intravenous ratio of 1:1. This can be done because of the high bioavailability of MMF. The oral formulation of MMF is available in 250 mg capsules. The prescribed dose will be rounded to the nearest dose possible with these capsules. Both intravenous and oral doses of MMF will be calculated using adjusted ideal body weight. If actual body weight is less than ideal body weight, actual body weight will be used.

Mini-Methotrexate 1/18/02, 1/15/03

Day +1 10 mg/m^2 IV 24 hrs after PBSC infusion is complete

Day +3 5 mg/m^2 IV

Day +6 5 mg/m^2 IV

8.3.3 ***GVHD PROPHYLAXIS REGIMEN C: Prograf, Sirolimus and mini-methotrexate***

- Prograf 0.02 mg/kg/d CI V, beginning day –3
- Sirolimus 12 mg PO loading dose on day –3 followed by 4 mg PO daily beginning on day –2
- Methotrexate 5mg/m^2 iv on day +1, +3, and + 6

11/18/04 1/6/05

Medications will be dosed based on adjusted ideal body weight

Prograf will be administered intravenously at a dose of 0.02 mg/kg q 24h by continuous infusion starting on Day –3. Intravenous tacrolimus will be discontinued once the patient starts eating and the drug will then be given orally at a dose of approximately 3 times the intravenous dose.

Dose Modifications of Prograf.

Suggested Prograf Dose Adjustment Guidelines

Plasma levels	Toxicity Grade	Tacrolimus Dose
<5 ng/ml	0	Increase 25%
5-20 ng/ml	0,1	No change
"	II	Decrease 25%
"	III	Decrease 50%
"	IV	Stop 100%
>20 ng/ml	0	Decrease 25%

		every 3-4 days

Drug Interactions

Drugs that may <u>increase</u> tacrolimus levels: Fluconazole, Itraconazole, Ketoconazole, erythromycin, H2 blockers, Verapamil, Diltiazem, nicardipine, danazol, bromocriptine, metoclopramide, methylprednisolone, somatostatin (octreotide).

Drug that may <u>decrease</u> tacrolimus levels: Rifampin, Phenobarbital, phenytoin, carbamazepne, Octreotide (may lower serum levels by decreasing intestinal absorption of the oral drug).

Drugs that may result in additive nephrotoxicity: Aminoglycosides, Amphotericin B, Acyclovir, Furosemide, TMP-SMX.

Sirolimus

<u>Sirolimus Dosing</u>

For adults, sirolimus will be administered at 12 mg orally loading dose on day –3, followed by 4 mg orally single morning daily dose (target serum level 3-12 ng/ml by HPLC). For Pediatric patients <40 kg, sirolimus will be administered at 3 mg/m^2 orally on day –3, followed by 1 mg/m^2 orally single morning daily dose. Dosing of sirolimus will be based on adjusted ideal body weight. If actual body weight is less than ideal body weight, actual body weight will be used.

11/18/04

<u>Sirolimus Dose Modifications</u>

Dose adjustments are based on clinical toxicity, blood levels, GVHD and clinical judgement involving the rate of rise or decline of the serum level. For levels <3 ng/mL, it is suggested that the dose be increased by approximately 25% increments no more frequently than every 2 days, rounded to the nearest full milligram until the target range is achieved. Conversely, for levels >12 ng/mL, it is suggested that the dose be decreased by approximately 25% no more frequently than every 2 days until the target level is achieved. If vomiting occurs within 20 minutes of administration the dose should be repeated. Anti-nausea medication may be given as needed. Sirolimus treatment may be ***discontinued*** **or** ***interrupted*** at any time for patient refusal to continue treatment or drug related SAE. The most serious reported SAE's from sirolimus in previous clinical studies after HSCT included hyperlipidemia, hypertension, rash, thrombocytopenia, leukopenia, arthralgias,

diarrhea and HUS. For management of cytopenias, it is recommended that patients receive growth factors or transfusions if necessary. For patients with HUS, sirolimus should be adjusted to maintain therapeutic levels; discontinuation of tacrolimus is recommended in this setting, particularly for cases requiring hemodialysis, seizures or severe hemolysis. No dose modification is recommended for hyperlipidemia; lipid-lowering agents should be instituted, with careful attention to the occurrence of rhabdomyolysis when HMGCoA reductase inhibitors are used. Sirolimus may be re-instituted if, in the judgment of the investigator, the primary clinical cause(s) for dose adjustment have been resolved.

Sirolimus treatment may also be interrupted for inability to swallow due to stomatitis; for patients with severe stomatitis who may not be able to resume oral intake in several days, mycophenolate mofetil (MMF) should be started as GVHD prophylaxis, at a dose of 15 mg/kg intravenously twice a day, until oral intake resumes, when sirolimus may substitute MMF.

Sirolimus should be adjusted for hepatic impairment, as follows: for bilirubin >2.0, reduce maintenance dose by 30%.

Sirolimus Serum Levels

Sirolimus trough levels should be measured at least weekly during the first 50 days post transplant. Levels of 3-12 ng/ml are considered therapeutic. Trough levels should be measured 20-24 hours after the last dose.

Drug Interactions

Sirolimus is a substrate for both cytochrome CYP3A4 and P-glycoprotein. Simultaneous administration with cyclosporine capsules

Methotrexate

Day +1 5 mg/m^2 IV starting 24 hours after the HSC infusion is complete
Day +3 5 mg/m^2 IV starting 72 hours after the HSC infusion is complete
Day +6 5 mg/m^2 IV

Leucovorin rescue may be used per discretion of primary Hematologist.

8.4 Treatment of GVHD

Diagnosis of Acute GVHD. Acute GVHD generally develops at time of engraftment or within the first three months after marrow transplantation. It is a clinicopathological syndrome involving the skin, the liver, and the gut. Serial biopsies and observations are used to help establish the diagnosis and severity of GVHD. The clinical appearance of skin GVHD can be mimicked by toxicity of the conditioning regimen and by drug reactions; therefore, documentation by skin biopsy should be performed when possible. Hepatic GVHD cannot be assessed solely on clinical grounds in patients who have concurrent drug toxicity, viral hepatitis, or toxicity caused by the conditioning regimen, and liver biopsy is helpful when possible. Gastrointestinal GVHD can be difficult to distinguish from infectious enteritis, and endoscopic biopsy should be done whenever possible. Appendix D presents a commonly used staging and grading system for acute GVHD.

Indications for Initial Treatment of Acute GVHD. In general treatment for GVHD should be started when Grade II GVHD criteria have been met. Grade II GVHD is moderately severe disease and usually consists of multiorgan disease. The decision to treat is based on the clinical assessment of the patient's condition, the onset time relative to the day of transplant, and the rapidity with which symptoms progress.

Agents to be Used for Initial Treatment of Acute GVHD. Acute GVHD should be treated with a corticosteroid-containing regimen. Methylprednisolone can be administered at a dose of at least 1 mg/kg body weight every 12 hours for 10-12 days. Tapering should begin between 10-20 days after starting initial treatment; the tapering of steroids can be determined by the institution.

New investigational agents for treatment of GVHD may become available; patients on this study are not routinely allowed to participate on other studies of drugs being evaluated for GVHD prophylaxis.

Patients who have received both methylprednisone and cyclosporine and have failed to respond may be treated with salvage regimens per institutional protocols. Salvage regimens may include antithymocyte globulin, anti-T cell antibodies, or psoralen and ultraviolet A irradiation (PUVA).

Diagnosis of Chronic GVHD. Manifestations of chronic GVHD typically do not occur until one to three months after marrow transplantation. The clinico-pathological classification of chronic GVHD is shown is Appendix 14.5. The patients will be characterized as either limited or extensive

chronic GVHD. Biopsies of the liver are recommended for patients who present with abnormal liver function tests in the absence of overt involvement of the skin, eyes, or mouth. Endoscopy and biopsy is recommended to establish gut involvement.

Agents to be used for Treatment of Chronic GVHD. Patients diagnosed with extensive chronic GVHD should be treated for 9-12 months with cyclosporine and prednisone per institutional protocol. Suggested dosages are prednisone at a dose of 1 mg/kg/day given as an oral dose in the morning. Cyclosporine may be administered at 6 mg/kg orally twice a day. FK-506 may be used instead of CSA . Thalidomide may also be used as allowed by FDA-reviewed protocols.

8.5 Use of Growth Factors.

Growth factors will not be used for 14 days post-transplant. After 14 days growth factor therapy may be administered at the discretion of the primary hematologist to patients with confirmed fungal infections or patients with systemic toxicities attributable to suspected or confirmed bacterial infections.

8.6 Graft Failure

Patients who have not engrafted by day 21 should undergo a bone marrow biopsy and aspirate, and chimerism studies should be performed. If the marrow cellularity is less than 5%, growth factor (G-CSF or GM-CSF) should be used. If there is no response in 7 subsequent days, graft failure is diagnosed.

8.7 Supportive Care

Patients will receive transfusions of blood products (platelets, RBC), antimicrobial drugs, nutritional support and other supportive measures as determined by their needs and according to the accepted standards of competent medical care. Patients should be given prophylaxis for Pneumocystis carinii, Herpes simplex, and fungal infections, administered according to institutional guidelines. HIV negative and hepatitis Bs Ag negative blood products (as determined by currently available serologic testing) will be used at all times. All blood products will be irradiated ($\geq$1500cGy) to circumvent the risk of GVHD caused by contaminating lymphocytes in the transfused fractions. Standard blood bank procedures should be followed to decrease the risk of CMV exposure in patients who are CMV seronegative.

8.8 Relapse

Donor lymphocytes will be allowed to treat documented relapse, including molecular and cytogenetic relapse.

9.0 PATIENT EVALUATIONS

Donor and recipient data will be collected and maintained by the NMDP according to their usual procedures.

9.1 Baseline Donor Data

Baseline donor evaluation will be per NMDP criteria. HLA typing should be by high resolution molecular methods for both HLA-A,-B,-C, DRB1 and DQB1 alleles.

9.2 Baseline Recipient Data

a. documentation of diagnosis and risk assignment
b. complete history and physical exam, performance status, height, weight, and all prior treatments
c. CBC, platelet, differential
d. complete ABO, Rh, and HLA typing; HLA typing should be by high resolution molecular methods for both HLA-A,-B,-C, DRB1 and DQB1 alleles.
e. cardiac, hepatic, renal and pulmonary function results
f. chest and other x-rays as clinically indicated
g. coagulation profile (PT, PTT)
h. chemistries not limited to but must include TSP, albumin, SGOT, SGPT, LDH, alkaline phosphatase, total bilirubin, Ca^{++}, Phos, Mg, uric acid, glucose, BUN, creatinine
i. viral screen, including CMV, HIV, hepatitis B and C
j. unique marker studies for use to assess chimerism

9.3 PBSC

Data collected will include the following:

9.3.1 Apheresis product

a. total nucleated cell count
b. FACS analysis for enumeration of CD34+ and T cells
c. sterility and viability

9.4 Preparative Cytoreduction and Transplantation

9.4.1 Pre-administration of chemotherapy/radiation therapy

a. CBC, differential, platelet
b. chemistries not limited to but must include TSP, albumin, SGOT, SGPT, LDH, alkaline phosphatase, total bilirubin,

indirect bilirubin, Ca^{++}, Phos, Mg, uric acid, glucose, BUN, creatinine

9.4.2 During cytoreductive therapy
a. vital signs daily
b. toxicities

9.4.3 Following completion of cytoreductive therapy until engraftment is documented.
a. CBC, platelet, daily until WBC>500/µL
b. vital signs daily
c. adverse experience reporting

9.4.4 Immediately following infusion of PBSC
a. vital signs (BP, pulse, temperature)
b. adverse experience reporting

9.4.5 Daily until hospital discharge - toxicities

9.4.6 Weekly until hospital discharge
a. chemistries
b. cyclosporine/prograf levels
c. infection assessments
d. GVHD summary - grades II-IV acute GVHD should be biopsy documented

9.4.7 Thirty days post-transplant
a. CBC, platelet, differential
b. chemistries
c. disease status assessment
d. GVHD assessment, including treatment summary
e. toxicities and adverse events including infection assessment
f. transfusion assessments (packed RBC, platelets)
g. brief physical exam, performance status
h. days in hospital, day ANC 500, and 1000/µL, and day platelets 20,000 and 50,000/µL
i. assessment of chimerism
j. Bone marrow aspiration and biopsy to document remission for those with bone marrow involvement at transplant 8/6/02

9.4.8 Follow-up at three and six months post-transplant
a. disease status
b. CBC, platelet
c. date platelet recovery > 100,000/µL
d. hospitalizations
e. GVHD assessment, including treatment summary

f. adverse experiences, toxicities & infection
g performance status
h assessment of chimerism
i. bone marrow for CML, relapsed leukemia patients, or those with bone marrow involvement at SCT 8/6/02

9.4.9 Follow-up every 6 months for 2 years and then yearly after that until death.

a. disease assessment
b. GVHD assessment, including treatment summary
c. assessment of chimerism at 6 months; if > 90% are of donor origin, additional evaluations not required unless relapse.
d. CBC, platelet, differential
e. hospitalizations
f. adverse events & infection
g. Bone marrow aspiration and biopsy at one year after transplant 8/6/02

10.0 ASSESSMENTS

Patient evaluations will be directed toward the acquisition of data addressing the primary and secondary objectives of the study.

10.1 Primary Objectives

10.1.1 Engraftment will be monitored by measuring the duration and extent of myelosuppression as shown by the days until ANC recovery (500, 1000/μL) and unmaintained platelet recovery (20,000; 50,000; and 100,000/μL). The primary engraftment endpoint is defined as the first of three consecutive days on which the absolute neutrophil count is $\geq$ 500/μL.

Failure to engraft will be defined as either (a) ANC consistently <500/μL in patients who survive for at least 21 days after transplant with marrow aplasia (cellularity < 5% at day 21), and who do not respond to 7 subsequent days of growth factor (primary graft failure, day 28), or (2) documented engraftment followed by severe neutropenia (ANC <300/μL), marrow aplasia (<5% cellularity) or absence of donor cells in the marrow or blood as demonstrated by a chimerism assay, without subsequent improvement occurring either spontaneously or after growth factor by Day 180 post-transplant (secondary graft failure).

Patients who are deceased prior to day 28 post-transplant will be considered inevaluable for engraftment failure.

10.1.2 Acute GVHD will be summarized weekly after transplant while the patients are in hospital and at each follow-up visit. Clinical observations including the existence of a skin rash, diarrhea and jaundice will be recorded. Evidence of grade II-IV GVHD will be documented by biopsy, if feasible. Use of drugs to treat GVHD and increase in dosages of drugs will be recorded. Chronic GVHD will be graded at each follow-up visit until day 180, and then every six months for two years.

10.2 Toxicities and side effects which will be evaluated include, but are not limited to, veno-occlusive disease, infections, renal toxicity, hepatic toxicity, and pulmonary toxicity. Methods of assessment will include monitoring blood chemistries, including peak serum bilirubin and creatinine levels, the incidence and severity of infectious complications, the incidence and severity of adverse events, including veno-occlusive disease, the requirements for transfusion products and causes of treatment failures. All grade III and IV non-hematological adverse events and all infectious episodes will be graded and documented. Toxicities will be recorded up to day 100 post transplant. Hepatic, pulmonary, and renal toxicities and infectious disease episodes will be summarized at the three and six month follow-ups. 11/18/04

Disease free survival will be monitored for two years post-transplant. New malignancy, lymphoproliferative or myeloproliferative disorders will be recorded.

11.0 ADVERSE EVENT REPORTING

11/18/04

This study will utilize the CTC (NCI Common Toxicity Criteria) Version 2.0 for toxicity and adverse event reporting. A copy of the CTC Version 2.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Definition of Adverse Events (AE):
An AE is defined as any untoward medical occurrence in a patient after administration of a study drug and/or procedure. An AE does not necessarily have a causal relationship with the use of a study drug and/or procedure. An AE can therefore be any unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a study drug and/or procedure, whether or not related to the study drug and /or procedure. Attempt will be made to distinguish AEs that are probably the result of GVHD versus AEs that are possibly or probably related to study drugs and/or procedures.

Attribution Categories:
Definite- The adverse event is clearly related to the treatment
Probable- The adverse event is likely related to the treatment

Possible- The adverse event may be related to the treatment
Unlikely- The adverse event is doubtfully related to the treatment
Unrelated- The adverse event is clearly NOT related to the treatment

Definition of Serious Adverse Event (SAE):
For reporting purposes, a serious adverse event (SAE) is any adverse event (AE) that is:

a) life threatening,
b) results in death,
c) requires hospitalization or prolongs an existing hospitalization,
d) results in persistent or significant disability,
e) causes a congenital birth defect,
f) causes cancer, or
g) is the result of a dosing error
h) certain medical events that may not result in death, be life-threatening, or require hospitalization, may also be considered a serious adverse event when medical or surgical intervention is necessary to prevent one of the outcomes listed above, as determined by appropriately trained personnel.

An SAE occurring in this study which meets the above definitions and is $\geq$ grade 3 and possibly, probably, or definitely related to the study procedure, or $\geq$ grade 4 regardless of the attribution must be reported until day 100.

SAE expedited reporting is not required for hematologic AEs or $\leq$ G3 AEs due to the following transplant procedure-related morbidities; nausea and vomiting, diarrhea, mucositis, hemorrhagic cystitis, hepatitis, or graft versus host disease in its various organ manifestations. 1/6/05

Serious Adverse Event (SAE) Reporting: All reports of SAEs occurring in City of Hope subjects must include a completed City of Hope Adverse event Report (COH AER) form (http://resadmin.coh.org/doc/irb3820.doc) that contains a narrative describing the event.

12.0 STATISTICAL CONSIDERATIONS

Patients receiving myeloablative therapy will be evaluated separately from the patients receiving reduced-intensity myeloablative therapy. The main objective is the estimation of the incidence of engraftment failure. The secondary objectives include evaluating toxicities, the rate of acute and chronic GVHD and relapse-free survival. 8/6/02

12.1 One hundred and thirty patients in each subgroup are sufficient to estimate the failure to engraft rate to within 9% (95% CI), and provide 80% power to detect an engraftment failure rate less than 10%, when the true failure rate is less than 5% (χ^2-test). There is less than a 10% one-sided

probability of concluding the failure rate is less than 10% when the true failure rate is 10% (1-sided α=0.1).

12.2 The accrual rate to this study is expected to be approximately 40 patients per year, with a 1:1 ratio between the myeloablative and reduced-intensity myeloablative regimens. The expected length of accrual is 6.5 years. 8/6/02

12.3 With 130 evaluable patients in each group, any toxicity occurring with at least a 4% probability is likely to be seen at least once (99% probability).

12.4 Survival will be calculated from date of transplant. Relapse-free, disease-free, and overall survival will
be estimated using Kaplan-Meier statistics.

13.0 ADMINISTRATIVE CONSIDERATIONS

13.1 Adverse Experiences

Serious adverse events (SAE) must be recorded and reported to the COH IRB and DSMB by the Investigator. A full report, including clear photocopies of hospital records, consultants' reports, autopsy findings here appropriate, and a summary of the outcome of the reaction by the investigator. 11/18/04

13.2 Criteria for Removal from Study

13.2.1 Death

13.2.2 Patient refused to continue

13.2.3 Grade 4 non-hematologic toxicity related to the administration of cells.

13.2.4 Impression or opinion of principal investigator that continuation is not in the best interest of the patient.

13.3 Institutional Review

Prior to implementation of this study, the research protocol and the proposed patient consent form must be reviewed and approved by a properly constituted Institutional Review Committee.

13.4 Informed Consent

Written consent will be obtained from each patient prior to entering the trial and will become part of the patient's permanent study record. Each

patient will be assured that study participation is voluntary and that he/she may withdraw at any time, without penalty.

At the time of obtaining written consent, the investigator will advise patients of the experimental nature of the study, the duration of the trial, alternate modes of treatment, and prevalent adverse reactions, which might occur. The patient's signature is to be witnessed. Parents or legal guardians of minor donors/recipients must provide fully informed consent for their children.

14.0 WOMEN AND MINORITIES GUIDELINES

All eligible patients from both genders and from all racial/ethnic groups will be recruited equally into this trial, with the only exclusionary criteria being those stated in Section 5.0. Based on our patient populations and previous experience with BMT for advanced hematological malignancies, the anticipated rates of entry into this study by gender and race/ethnicity are as follows:

Race/Ethnicity by Gender for Advanced Malignancy Patients Receiving BMT at City of Hope

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or unknown
Female	0%	8%	1%	32%	58%	1%
Male	<1%	8%	3%	33%	54%	3%
Total	<1%	8%	2%	33%	55%	2%

15.0 REFERENCES

(1) Bensinger WI, Price TH, Dale CD, et al. The effects of daily recombinant human granulocyte colony stimulating factor administration on npormal granulocyte donors undergoing leukapheresis. Blood 1993; 81:1883.

(2) Bensinger W, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 2001; 344:175-181.

(3) Sierra J, Storer B, Hansen JA, et al. transplantation of marrow cells from unrelated donors for treatment of high-risk acute leukemia: The effect of leukemic burden,donor HLA matching, and marrow cell dose. Blood 1997; 89:4226-4235.

(4) Ottinger HD, Beelen DW, Scheulen B, et al. Improved immune reconstitution after allotransplantation of peripheral blood stem cells instead of bone marrow. Blood 1996; 88:2775-2779.

(5) Dreger P, Haferlach T, Eckstein V, et.al. G-CSF mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft. Br J Haematol 1994; 87:609.

(6) Lane TA, Law P, Maruyama M, Young D, et.al. Harvesting and enrichment of hematopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF: potential role in allogeneic marrow transplantation. Blood 1995; 85:275.

(7) Schmitz N, Dreger P, Suttorp M, et.al. Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). Blood 1995; 85:1666.

(8) Urbano-Ispizua A, Solano C, Brunet S, et.al. Allogeneic peripheral blood progenitor cell transplantation: analysis of short-term engraftment and acute GVHD incidence in 33 cases. Bone Marrow Transplant 1996; 18:35.

(9) Brown RA, Adkins D, Goodnough LT, et.al. Factors that influence the collection and engraftment of allogeneic peripheral blood stem cells in patients with hematologic malignancies. J Clin Oncol 1997; 15:3067.

(10) Bensinger W, Weaver C, Appelbaum FR, et.al. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood 1995; 85:1655.

(11) Brown RA, Adkins D, Khoury H, et.al. Long-term follow-up of high-risk allogeneic peripheral blood stem cell transplant recipients: Graft-versus-host disease and transplant-related mortality. J Clin Oncol 1999; 17:806.

(12) Chao NJ, Schmidt G, Niland JC, Amylon MD, et.al. Cyclosporine,methotrexate,and prednisone compared with cyclosporine and prednisone for prophylaxis of acute graft-versus-host disease. N Engl J Med 1993; 329:1225.

(13) Nash RA, Etzioni R, Storb R, et al. tacrolimus (FK506) alone or in combination with methotrexate or methylprednisolone for the prevention of acute graft-versus-host disease after marrow transplantation from HLA-matched siblings: A single-center study. Blood 85, 3746. 1995.

(14) Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (Prograf,FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA identical sibling bone marrow transplantation. Blood 92, 2303-2314. 10-1-1998.

(15) Nash RA, Antin J, Karanes C, Fay JW, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine (CSP) for prophylaxis of acute graft-versus-host disease (GVHD) after marrow transplantation from unrelated donors. Blood (Suppl) 90, 561a. 1997.

(16) Yu C, Siedel K, Nash RA, Deeg HJ, et al. Synergism between mycophenolate mofetil and cyclosporine in preventing graft-versus-host disease among lethally irradiated dog given DLA-nonidentical unrelated marrow grafts. Blood 1998; 91:2581-2587.

(17) Bornhauser M, Schuler U, Porksen G, et al. Mycophenolate mofetil and cyclosporine as graft-versus-host isease prophylaxis after allogeneic blood stem cell transplantion. Transplantation 1999; 67:499-504.

(18) Bensinger WI, Clift R, Martin P, et al. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: A retrospective comparison with marrow transplantation. Blood 1996; 88:2794-2800.

(19) Schmitz N, Bacigalupo A, Hasenclever D, et.al. Allogeneic bone marrow transplantation vs. filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukemia: first results of a randomized multicenter trial of the European Group for Blood and Marrow transplantation. Bone Marrow Transplant 1998; 21:995.

(20) Champlin RE, Schmidt N, Horowitz MM, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells fo allogeneic transplantation. Blood 2000; 95:3702-3709.

(21) Davies SM, Kollman C, Anasetti C, et al. Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the National Marrow Donor program. Blood 2000; 96:4096-4102.

(22) McGlave PB, Shu XO, Wen W, et al. Unrelated donor marrow transplantation for chronic myelogenous leukemia: 9 years' experience of the National Marrow Donor Program. Blood 95, 2219-2225. 4-1-2000.

(23) Ringden O, Runde V, Hagglund H, et al. Transplantation of peripheral blood progenitor cells from unrelated donors. Transplantation 1998; 66:142-143.

(24) Ringden O, Remberger M, Runde V, et al. Faster engraftment of neutrophils and platelets with peripheral blood stem cells from unrelated donors: A comparison with marrow transplantation. Bone Marrow Transplant 2000; 26 Suppl 2:S6-S8.

(25) Anasetti C, Amos D, Beatty PG, et al. Effect of HLA Compatibility on Engraftment of Bone Marrow Transplants in Patients with Leukemia or Lymphoma. N Engl J Med 1989; 320:197-204.

(26) Petersdorf EW, Longton GM, Anasetti C, et.al. Association of HLA-0C disparity with graft failure after marrow transplantation from unrelated donors. Blood 1997; 89:1818-21823.

(27) Beatty PG, Clift RA, Mickelson EM, et al. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med 1985; 313:765-771.

(28) Servida P, Gooley T, Hansen JA, et.al. Improved survival of haploidentical related donor marrow transplants mismatched for HLA-A or B versus HLA-DR. Blood (Suppl) 88, 484a. 1996.

(29) Kernan NA, Bartsch G, Ash RC, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National marrow donor program. N Engl J Med 1993; 328:593-602.

(30) Petersdorf EW, Longton GM, Anasetti C, et al. The significance of HLA-DRB1 matching on clinical outcome after HLA-A,B,DR identical unrelated donor marrow transplantation. Blood 1995; 86:1606-1613.

(31) Petersdorf EW, Mickelson EM, Anasetti C, et al. Effect of HLA mismatcjes on the outcome of hematopoietic transplants. Curr Opin Immunol 1999; 11:521-526.

(32) Sasazuki T, Juji T, Morishima Y, et.al. Effect ofmatching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. The New England Journal of Medicine 339, 1177-1185. 1998.

APPENDICES

A Pharmaceutical Information
B Methotrexate and Cyclosporine Dose Modification Guidelines
C Total Body Irradiation
D GVHD Assessment
E Performance Status
F Study Calendar
G IV Busulfan Kinetics
H CTC Version 2.0

APPENDIX A

PHARMACEUTICAL INFORMATION

1.0 G-CSF (NEUPOGEN®)

GENERAL:

Recombinant Human Granulocyte Colony Stimulating Factor (rHu-G-CSF, E. coli./CSF, Neupogen®, Amgen, Thousand Oaks, CA) is a growth factor that stimulates proliferation and differentiation of committed granulocytic progenitor cells and enhances the number of circulating progenitor cells.

ADMINISTRATION:

G-CSF is available in single use vials of preservative-free solution containing 300 mg/mL of material (1 mL vial = 300 mg and 1.6 mL vial = 480mg). The donor will receive G-CSF, 10 □g/kg/day, by subcutaneous injection for 5-10 days with day 5 being the first day of leukapheresis.

TOXICITY:

The major expected toxicity is bone pain which can be treated successfully with analgesics. Fever, flu-like symptoms, pleuritis, pericarditis and other systemic reactions may occur with G-CSF, usually at high dosages. Reversible and mild elevation in uric acid, LDH, alkaline phosphatase have been noted. Transient decreases in blood pressure, not requiring treatment were noted in 4% of patients in one trial of G-CSF. Allergic toxicities have rarely been observed, at a rate of approximately one in one hundred thousand administrations.

2.0 TOTAL BODY IRRADIATION

TOXICITIES:

Gastrointestinal: virtually all patients will experience nausea and vomiting after irradiation. This can be diminished somewhat with antiemetics. Most patients develop some diarrhea in the first week post irradiation. This can be treated symptomatically

Parotitis: some patients will experience symptomatic parotitis within the first 24 hours post irradiation. This resolves spontaneously over several days.

Fever: significant fever (greater than 38□C) is usual for 24 hours post irradiation. This can be treated symptomatically.

Skin: erythema may occur in patients within 24 hours and resolve in 2-3 days. Most patients will get some degree of hyperpigmentation within 2-3 weeks of transplantation.

Mucositis - most patients will develop moderate to severe mucositis of the oral and GI tracts, which will be managed with aggressive nursing mouth care and prophylactic antifungal and antiviral agents.

LATE EFFECTS:

There is the possibility of cataract formation. Although mild cataracts may occur in up to 30-50% of cases with single dose TBI, cataracts have been seen in <10% of patients treated with hyperfractionated TBI.

There is a possibility that secondary malignancies may develop, particularly due to the combined effects or irradiation and an alkylating agent (cyclophosphamide). Lung fibrosis may also develop from frequent use of chemotherapy and irradiation.

Sterility may result following total body irradiation and administration of alkylating chemotherapy (cyclophosphamide); the risk increases with the number of years since puberty.

Hypothyroidism has been reported in small numbers of adults routinely monitored post transplant with hormonal replacement as indicated.

3.0 CYCLOPHOSPHAMIDE (CYTOXAN®, NEOSAR®)

GENERAL:

Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. It is biotransformed principally in the liver to active alkylating metabolites. These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. The mechanism of action is thought to involve cross-linking of DNA. It is eliminated primarily in the form of metabolites, but from 5-25% of the drug is excreted in the urine unchanged.

ADMINISTRATION:

Lyophilized cyclophosphamide (Cytoxan™) for injection contains 75 mg mannitol per 100 mg cyclophosphamide (anhydrous) and is supplied in

vials for single-dose use (100, 200 and 500 mg vials). It should be reconstituted by adding Sterile Water for Injection, USP, to the vial and shaking to dissolve.

TOXICITIES:

Hematologic: myelosuppression [leukopenia (nadir 8-14 days), thrombocytopenia, anemia]. Hematocrit decrements out of proportion to cessation of production will occur at this dose, presumably due to hemolysis.

Urinary: hemorrhagic cystitis (patients must be well hydrated before, during, and after treatment and have adequate renal function). Hematuria is not uncommon at this dose but is usually not symptomatic or severe unless there is inadequate diuresis. An occasional patient will get severe cystitis despite adequate urine flow. Pyridium and/or continuous bladder irrigation will give symptomatic relief.

Gastrointestinal: nausea, vomiting, anorexia and, less frequently, abdominal discomfort or pain and diarrhea may occur. Variable combinations of antiemetics usually provide significant symptomatic relief.

Cardiac: cardiotoxicity has been observed in patients receiving high doses of cyclophosphamide. At doses greater than 7.6 g/m^2, clinical heart failure and fatal cardiac necrosis have been observed.

Fluid retention: Cyclophosphamide has an anti-diuretic effect usually counteracted by furosemide administration. Careful physical examination should be made and accurate weights should be determined to detect fluid overload early.

Pulmonary: High dose cyclophosphamide is associated with significant pulmonary toxicity characterized by dyspnea, decreased pO_2, pulmonary infiltrates and occasional effusions. This can be prevented and/or reversed by use of corticosteroids.

Skin: Alopecia occurs commonly and is reversible. Hair may be different in texture or color. Skin rash occurs occasionally, and pigmentation of the skin and changes in nails can occur.

Other: sterile phlebitis, gonadal abnormalities, teratogenicity. With too rapid IV push, oropharyngeal tingling, "metallic" taste, headache, urticaria, and facial flushing can occur.

4.0 BUSULFAN (MYLERAN)

MECHANISM OF ACTION:

Busulfan is a bifunctional alkylating agent. In aqueous media, busulfan hydrolyses to produce reactive carbonium ions that can alkylate DNA.

Formulation and stability: Busulfan injection is a sterile, pyrogen-free solution provided in a mixture of dimethylacetamide (DMA) and polyethlyeneglycol 400 (PEG 400). It is supplied in 10-ml single use ampules at a concentration of 6-mg Busulfan per ml. Each ampule contains 60mg of Busulfan in 3.3 ml of DMA and 6.7 ml of PEG 400. When diluted in normal saline or D5W to a concentration of 0.5mg/mL, the resulting solution must be administered within eight (8) hours of preparation; including the 2 hours of infusion of the drug.

Stable at 4º C for at least twelve months. Ampules should be stored refrigerated at 2-8º C. Do not freeze. Ampules may be stored for up to seven days at room temperature.

Solution preparation: prepare the Busulfan solution as follows: Use sterile, non-pyrogenic, disposable containers, syringes, needles, stopcocks, and transfer tubing, etc. Calculate the amount of drug to be administrated based on the dosage and the patient's body weight.

Prepare a solution of 0.9% sodium chloride injection USP (normal saline) calculated Busulfan dose in ml from the step above.

Break off the top of the ampule and remove the calculated volume of Busulfan from the container by using a syringe fitted with a filter needle or equivalent. Transfer the contents of the syringe into the calculated amount of either normal saline or D5W making sure that the drug flows into and through the solution. Mix by inverting the bag.

ADMINISTRATION:

Each dose of the drug will be given by slow central intravenous infusion over 2 hours. Caution: Do not administer as an intravenous push or bolus.

SUPPLIER:

This drug is commercially available; manufactured by Orphan Medical Inc.

TOXICITIES:

Toxicity from busulfan includes:

a). Severe bone marrow hypoplasia, which would be fatal without administration of bone marrow, stem cells.

b). Nausea and vomiting which can be decreased by the use of sedation and anti-emetics.

c). Stomatitis and diarrhea which can be treated symptomatically with fluid replacement and atropine or diphenoxylate H C1.

d). Pulmonary fibrosis characterized by delayed onset of cough, shortness of breath and low-grade fever.

e). Hepatic damage, which can occur in combination with cytoxan or as a single agent and can result in significant hepatic toxicity which, can be fatal.

f). Temporary hyperpigmentation of the skin and nail bed changes.

g). Grand mal seizures which can be prevented by the prophylactic administration of Dilantin.

5.0 MELPHALAN (L-phenylalanine mustard, L-PAM, Alkeran)

FORMULATION:

Melphalan is supplied as a freeze-dried powder. Each single-use vial contains melphalan hydrochloride equivalent to 50mg of melphalan and 20mg povidone. Reconstitute with the sterile diluent provided. Each vial of diluent contains sodium citrate 0.2g, propylene glycol 6ml, ethanol (96%) 0.52ml and water for injection to total 10ml.

PREPARATION FOR USE:

Reconstitute each 50ml vial by rapidly injecting 10ml diluent provided. Immediately shake vial vigorously until a clear solution is obtained. This provides a 5mg/ml solution.

STORAGE AND STABILITY:

Intact vials are stored at room temperature (15-30°C) protected from light. Once reconstituted with the diluent provided, the solution is chemically and physically stable at room temperature for 90 minutes. Melphalan, when reconstituted with the diluent provided and subsequently diluted in NS to a concentration of 0.1-0.45mg/ml is chemically and physically stable for 60 minutes at room temperature. Do not refrigerate.

ADMINISTRATION:

Reconstituted, undiluted melphalan has been administered via

central line using doses of 70-200mg/m^2 over 2 to 20 minutes.

6.0 MESNA (NSC-113891)

MODE OF ACTION:

Sodium-2-mercapto-ethanesulfonate (Mesna) is a urothelial protectant. It binds to acrolein, the urotoxic metabolite of ifosfamide, and also inhibits the breakdown of its 4-hydroxy metabolites. It is excreted exclusively in the urine.

SUPPLY, RECONSTITUTION AND ADMINISTRATION:

Mesna is available from Bristol-Meyer Squibb as a l00 mg/ml solution (400 mg/4 ml ampule). It may be administered intravenously by bolus an/or continuous infusion.

TOXICITIES:

Nausea and vomiting are the only side effects observed with Mesna.

7.0 METHOTREXATE

GENERAL:

Methotrexate is an antimetabolite that binds to dihydrofolic acid reductase, thereby preventing the reduction of folic acid to tetrahydrofolate. It interferes with DNA synthesis, repair, and cellular replication. Actively proliferating tissues are in general more sensitive to this effect.

ADMINISTRATION:

Methotrexate LPF® Sodium (methotrexate sodium injection), Isotonic Liquid, Preservative Free, for single use only, is available in 25 mg/mL, 2 mL, 4 mL, 8 mL, and 10 mL vials, containing 50 mg, 100 mg, 200 mg, and 250 mg of methotrexate respectively. If desired, the solution may be further diluted immediately prior to use with an appropriate sterile, preservative-free medium.

Methotrexate Sodium for Injection, Freeze Dried, Preservative Free, Low Sodium, for single use only, is available in 20 mg, 50 mg, and 1 gram vials, containing approximately 0.14, 0.33 and 7 mEq of sodium respectively. Reconstitute immediately prior to use with an appropriate sterile, preservative-free medium.

Methotrexate Sodium Injection, Isotonic Liquid, Preservative Protected, is available in 25 mg/mL, 2 mL (50 mg), and 10 mL (250 mg) vials. The preservative formulation contains benzyl alcohol and must not be used for intrathecal or high dose therapy. If desired, the solution may be further diluted with a compatible medium.

TOXICITIES:

Hematologic: myelosuppression [leukopenia (nadir 7 days), thrombo-cytopenia, anemia].

Hepatic: acute (elevated transaminases) and chronic (fibrosis and cirrhosis) hepatic toxicity. Chronic toxicity has generally occurred after prolonged use (generally 2 years or more) and after a total dose of at least 1.5 grams.

Urogenital: severe nephropathy or renal failure, azotemia, cystitis, hematuria; defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction and vaginal discharge; infertility, abortion, fetal defects. Close attention to renal function including adequate hydration, and urine alkalinization are essential for safe administration.

Gastrointestinal: gingivitis, pharyngitis, stomatitis, anorexia, nausea, vomiting, diarrhea, hematemesis, melena, gastrointestinal ulceration and bleeding, enteritis. Should be used with extreme caution in the presence of peptic ulcer disease or ulcerative colitis. Therapy may be discontinued if ulcerative stomatitis or other severe GI adverse reactions occur.

Pulmonary: interstitial pneumonitis deaths have been reported, and chronic interstitial obstructive pulmonary disease has occasionally occurred. Pulmonary symptoms or a nonspecific pneumonitis may be indicative of a potentially dangerous lesion and require interruption of treatment and careful investigation; infection needs to be excluded. This lesion can occur at all dosages.

Skin: erythematous rashes, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis.

Central Nervous System: headaches, drowsiness, blurred vision. There have been reports of leukoencephalopathy following intravenous administration of methotrexate to patients who have had craniospinal irradiation. Aphasia, hemiparesis, paresis, and convulsions have also occurred following administration of methotrexate. Following low doses,

occasional patients have reported transient subtle cognitive dysfunction, mood alteration, or unusual cranial sensations.

Other: opportunistic infection, arthralgia/myalgia, loss of libido/impotence, diabetes, osteoporosis and sudden death. A few cases of anaphylactoid reactions have been reported.

8.0 CYCLOSPORINE (SANDIMMUNE® OR EQUIVALENT, NEORAL®)

GENERAL:

Cyclosporine is a cyclic polypeptide immunosuppressant agent consisting of 11 amino acids. It is a potent immunosuppressant and has been demonstrated to suppress some humoral immunity and to a greater extent, cell-mediated reactions such as allograft rejection and GVHD in many animal species for a variety of organs. Evidence suggests that the effectiveness is due to specific and reversible inhibition of immunocompetent lymphocytes in certain phases of the cell cycle; T lymphocytes are preferentially inhibited.

ADMINISTRATION:

Sandimmune® Soft Gelatin Capsules are supplied 25, 50 or 100 mg capsules in unit dose packages of 30 capsules. Should be stored in the original unit-dose container at temperatures below 86ºF (30ºC).

Sandimmune® Oral Solution is supplied in 50 mL bottles containing 100 mg of cyclosporine per mL. Should be stored in the original container at temperatures below 86ºF (30ºC). Do not store in the refrigerator. Once opened, the contents must be used within 2 months.

Sandimmune® Injection (cyclosporine concentrate) for intravenous infusion is supplied as a 5 mL sterile ampule containing 50 mg of cyclosporine per mL. Should be stored at temperatures below 86ºF and protected from light and freezing. Immediately before use, the i.v. concentrate should be diluted 1 mL Sandimmune injection in 20-100 mL with an appropriate sterile preservative free media and given in slow intravenous infusion over approximately 2-6 hours. Diluted infusion solutions should be discarded after 24 hours.

Neoral ® Soft Gelatin Capsules are supplied as 25 and 100 mg capsules. Neoral® has greater bioavailability than Sandimmune form. Less Neoral® is needed to yield the same blood concentration derived from Sandimmune.

Neoral® Oral Solution is supplied in 50mL bottles. Neoral® has greater bioavailability than Sandimmune form. Less Neoral® is needed to yield the same blood concentration derived from Sandimmune.

TOXICITIES:

Hematologic: leukopenia, thrombocytopenia, anemia; lymphoma

Urogenital: renal dysfunction. It is not unusual for serum creatinine and BUN levels to be elevated during cyclosporine therapy. Overt nephrotoxicity early after transplantation is characterized by rapidly rising BUN and creatinine. This form is usually responsive to dosage reduction. Mild nephrotoxicity, generally noted 2-3 months after transplant, is often responsive to dose reduction. A form of chronic progressive cyclosporine-associate nephrotoxicity characterized by serial deterioration in renal function and morphologic changes in the kidneys, will often fail to show a reduction in rising serum creatinine despite a decrease or discontinuation of cyclosporine administration.

Gastrointestinal: gum hyperplasia, oral thrush, diarrhea, nausea, vomiting, hepatotoxicity, and abdominal discomfort.

Pulmonary: sinusitis

Skin: hirsutism, acne.

Central Nervous System: tremor, convulsions, headache. Hypomagnesemia has been reported in some, but not all, patients exhibiting convulsions while on cyclosporine therapy.

Cardiovascular: hypertension (usually mild to moderate), cramps.

Other: sinusitis, flushing, increased low-density lipoproteins, A few cases of anaphylactoid reactions have been reported in patients receiving Sandimmune injection®, believed to be due to the Cremophor EL® used as the vehicle for the i.v. formulation. Closely monitor patients receiving i.v. infusions of the drug.

9.0 MYCOPHENOLATE MOFETIL (MMF) (CELLCEPT)

DESCRIPTION

MECHHANISM OF ACTION:

MMF is a morpholinoethyl ester of mycophenolic acid (MPA). A product of several penicillium species, MPA possesses antibacterial, antifungal,

antiviral, antitumor and immunosuppressive properties. MMF is a pro-drug since the immunosuppressive activity is evident only after hydrolysis to MPA [26]. MMF was developed to enhance the bioavailability of MPA. MPA mediates its effect by inhibiting inosine monophosphate dehydrogenase (IMPDH), an enzyme that catalyzes the oxidation of inosine monophosphate to xanthine monophosphate, an intermediate metabolite in the synthesis of guanosine triphosphate (GTP). Lymphocytes rely on the de novo purine synthesis pathway for the nucleotides necessary for DNA synthesis; other cells can also rely on the salvage pathway [5,6]. The action of MPA results in the depletion of the nucleotide pool in cell synthesis. An additional mode of action of MPA may be that, by depletion of GTP, it inhibits recruitment of leukocytes to sites of inflammation by inhibiting the glycosylation of lymphocyte glycoproteins that are involved in intercellular adhesion [27].

PHARMACOKINETICS:

MMF absorbtion 94%, rapidly absorbed and converted to MPA in the liver and undergoes enterohepatic recirculation; 97% bound to albumin. MPA is metabolized in the liver to an inactive metabolite, less than 1% is excreted in urine; half-life 18 hours and is not removed by hemodialysis.

FORMULATION:

Oral MMF supplied in 250 mg hard gelatin capsules and as a liquid suspension. Capsules can be stored at room temperature. A liquid suspension is also available (200 mg/ml, 175 ml bottle).

IV MMF: Intravenous: MMF for intravenous use will be supplied as a lyophilized powder. The intravenous formulation will be stored and prepared according to the drug company instructions. Appendix A describes formulation, preparation, reconstitution, administration, and precautions.

SUPPLIER:

This drug is commercially available

HUMAN TOXICOLOGY:

There have only been limited studies of MMF in patients after marrow transplantation. Previous clinical studies in patients after renal allografting suggested that the principal adverse reactions associated with the administration of MMF include GI: hemorhage (3%), diarrhea (16-36%), constitpation, dyspepsia, severe neutropenia (2%), leukopenia, sepsis, vomiting and possibly a higher incidence of certain viral infections (CMV,

VZV, Herpes Simplex). Commonly seen post-op day 30–180 increased incidence of lymphoma (1%) compared to azathioprine may have less myelosuppression than azathioprine due to its selective mechanism.

DRUG INTERACTIONS:

Anitacids decrease abosorbtion food decreases the peak concentration by 40%, but not the extent of absorbtion drugs that interfere with enterohepatic recirculation may decrease absorbtion, i.e cholestyramine

10.0 TACROLIMUS OR FK5O6 (PROGRAF®)

DESCRIPTION:

Prograf is available as and oral administration and a sterile solution (tacrolimus injection containing the equivalent of 5 mg anhydrous tacrolimus in 1ml for administration by IV infusion only. Each ml contains polyoxyl 60 hydrogenated caster oil (HCO-0), 200 mg, and dehydrated alcohol, USP, 80.0% v/v. Prograf injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection before use.

Tacrolimus, previously known as FK506, is active ingredient in Prograf. Tacrolimus is a macrolide immunosuppressant produced by *Streptomyces tsukubanensis*.

MECHANISM OF ACTION:

Tacrolimus prolongs the survival of the host and transplanted graft in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb. In animals, tacrolimus has been demonstrated to supress some humoral immunity and, to a greater extent, cell-mediated reactions such as allograft rejection, delayed type hypersensitivity, collagen induced arthritus, experimental allergic encephalomyelitis, and graft versus host disease.

HUMAN TOXICOLOGY:

Headache, tremor, insomnia, parathesia, diarrhea, nausea, constipation, LFT abnormality, anorexia, vomiting, hypertension, creatinine increased, BUN increased, UTI, oliguria, hyperkalemia, hypokalemia, hyperglycemia, hypomagnesemia, anemia, leukocytosis, abdominal pain, pain, fever, asthenia, ascites, peripheral edema, pleural effusion, atelectasis, dyspnea, pruritus, rash, renal. Increased susceptibility to infection and possible development of lymphoma.

KINETICS:

Tacrolimus activity is primarily due to the parent drug. The pharmacokinetic parameters of tacrolimus have been determined following IV and PO administration in healthy volunteers, liver transplant and kidney patients. Due to intersubject variability in tacrolimus pharmacokinetics, individualization of dosing regimen is necessary for optimal therapy. PK data indicate that whole blood concentrations rather than plasma concentrations serve as the more appropriate sampling compartment to describe tacrolimus pharmacokinetics

.

SUPPLIER:

This drug is commercially available

.

11.0 METHYLPREDNISOLONE (SOLU-MEDROL®)

GENERAL:

Methylprednisolone is a synthetic analog of the naturally occurring glucocorticoids, which have profound and varied metabolic effects including salt retention, anti-inflammatory properties and modification of the immune response. Methylprednisolone is a potent anti-inflammatory steroid with less tendency than prednisolone to induce sodium and water retention. Methylprednisolone sodium succinate (SOLU-MEDROL®) has the same metabolic and anti-inflammatory actions as methylprednisolone.

ADMINISTRATION :

Methylprednisolone sodium succinate is available in 40, 125, 500, 1000, and 2000 mg vials as a sterile powder which must be reconstituted before use. Use only the accompanying diluent or bacteriostatic water for injection with benzyl alcohol when reconstituting SOLU-MEDROL®.

TOXICITIES:

Metabolic: fluid and salt retention, glucosuria and hyperglycemia, obesity.
Gastrointestinal: peptic ulcer.
Neurologic: irritability, muscle weakness.
Cardiovascular: hypertension.
Other: hirsutism, osteoporosis, pancreatitis, cataracts and seizures (very rare).

12.0 FLUDARA®

(Fludarabine phosphate)

MECHANISM OF ACTION:

Fludarabine is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

PHARMACOLOGY:

Phase I studies in humans have demonstrated that fludarabine phosphate is rapidly converted to the active metabolite, 2-fluoro-ara-A, within minutes after intravenous infusion. In a study with 4 patients treated with 25mg/m^2/day for 5 days, the half-life of 2-fluoro-ara-A was approximately 10 hours. Approximately 23% of the dose was excreted in the urine as unchanged 2-fluoro-ara-A. The mean Cmax after Day 1 dose was 0.57 mcg/ml and after the Day 5 dose was 0.54 mcg/ml.

DOSE AND ADMINISTRATION:

The recommended dose of fludarabine is 25 mg/m^2 administered intravenously over a period of approximately 30 minutes daily for five consecutive days. Each 5 day course of treatment should commence every 28 days. The optimal duration of treatment has not been clearly established. The dose of fludarabine used in this protocol is nonmyeloablative, but does cause significant immunosuppression.

ADVERSE REACTIONS:

Hematopoietic Systems: Hematologic events (neutropenia, thrombocytopenia, and/or anemia). Life-threatening and sometimes fatal autoimmune hemolytic anemia have been reported.
Metabolic: Tumor lysis syndrome.
Nervous System: Objective weakness, agitation, confusion, visual disturbances, coma and peripheral neuropathy.
Pulmonary System: Pneumonia, dyspnea, cough and interstitial pulmonary infiltrate.
Gastrointestinal System: Nausea, vomiting, anorexia, diarrhea, stomatitis and gastrointestinal bleeding.
Cardiovascular: Edema
Genitourinary System: Rare cases of hemorrhagic cystitis.
Skin: Skin rashes.

PREPARATION OF SOLUTIONS:

Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH to 7.7. Fludarabine for injection should be prepared for parenteral use by aseptically adding 2ml of sterile water. Each ml of the resulting solution will contain 25 mg of fludarabine phosphate. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP.

STORAGE AND STABILITY:

Fludarabine should be stored under refrigeration, between 2º-8ºC. Fludarabine contains no antimicrobial preservative and thus should be used within 8 hours of reconstitution.

13.0 VP-16 (VP-16-213) (Etoposide) (Vepesid)

8/6/02

CHEMISTRY:

VP-16 is a semi-synthetic podophyllotoxin derivative from the plant podophyllum pletatum, has anti-neoplastic properties in experimental animals and in man. The empiric formula C_{29} H_{32} O_{13} has a molecular weight of 588.

MECHANISM OF ACTION:

The epipodophyllotoxin exert phase-specific spindle poison activity with metaphase arrest, but in contrast to the vinca alkaloids, have an additional activity of inhibiting cells from entering mitosis. Suppression of tritiated thymidine, uridine, and leucine incorporation in human cells in tissue culture suggest effects against DNA, RNA and protein synthesis.

ANIMAL TUMOR DATA:

Significant anti-tumor effect has been demonstrated in L-1210, mouse sarcoma 37 and 180, Walker carcinosarcoma and Erlich ascites tumor. With the L 1210 system, activity was schedule-dependent, having greater effect with a twice weekly administration than with daily dosing or the administration of single large doses. The drug is active given intraperitoneally or orally in L 1210. No effect was demonstrated intracerebrally inoculated L 1210.

ANIMAL TOXICOLOGY:

The predominant toxicities of VP-16 in animal studies involve the hematopoietic system, with toxicity to the liver and GI tract occurring only at doses producing profound myelosuppression. Anemia, leukopenia, and lymphoid involution occur in mice, rats and monkeys. Acute toxicity

investigations have been complicated by the toxicity of the solvent system. The LD-50 of the solvent plus drug approached that of the solvent alone. Immuno-suppressive effects occur with an inhibition of antibody production in mice and monkeys, and prevention of experimental allergic encephalomyelitis in rats (cell mediated immunity).

HUMAN TOXICOLOGY:

Reversible myelotoxicity has been uniformly observed to be the major toxicity of VP-16 and to represent the only clinically significant side effect. Following a single IV injection, peak myelotoxicity occurs at 7 to 9 days. Following daily IV injections for 5 to 7 days, myelotoxicity is maximal between 12 to 16 days from the initiation of therapy. Bone marrow suppression is mainly manifested as granulcytopenia with thrombocytopenia and anemia occurring to a lesser extent. Transient, modest nausea, usually without vomiting, is common. Occasional alopecia is reported. VP-16 does not produce stomatitis, phlebitis, neurotoxicity, hepato-toxicity or nephro-toxicity. Hypotension and anaphylaxis are occasional side effects. Hypotension can be managed by infusing the drug over at least a 30 minute period. Occasionally, fever may be a result of VP-16 administration.

PHARMACEUTICAL DATA:

Formulation: 100 mg of VP-16 is supplied as 5 ml of solution in clear ampules for injection. Each ampule also contains anydrous citric acid 10 mg, benzylalcohol 150 mg, polysorbate 80 purified 400 mg, polyethylene glycol and absolute alcohol. The contents of the ampule are non-aqueous and must be diluted with 20 to 50 volumes of sodium chloride injection USP. The time before precipitation depends on concentration.

Dilution	Time
1:20	30 minutes
1:50	3 hours
1:100	6 hours

Storage and stability: The drug is available as a box of 10 ampules that are stored at room temperature. Each ampule should be kept in the box to protect it from light. VP-16 is less stable in 5% Dextrose injection and precipitation is reported. VP-16has a minimum infusion time of 30 minutes to reduce hypotension.

SUPPLIER:

VP-16 is commercially available.

14. Sirolimus (Rapamune®)

14.1 Mechanism of action: see section 2.2

14.2 Toxicity: The most commonly reported toxicities are hyperlipidemia, arthralgia, cytopenias, epistaxis, edema and rash. Despite the similarity between sirolimus and tacrolimus, there is no neurotoxicity or nephrotoxicity with sirolimus because of its inability to inhibit calcineurin. Phase III clinical trials have indicated that the primary toxicities are hypertriglyceridemia, hypercholesterolemia, mild thrombocytopenia, anemia, leukopenia, hypokalemia, elevated LDH, arthralgia, epistaxis, edema, and infections. Clinically significant elevations in hepatic transaminases without sequelae were noted in the prior phase II study with this drug, however, the incidence of veno-occlusive disease of the liver was not noted to be higher than expected in trials of unrelated transplantation. A syndrome of thrombotic microangiopathy, comprised of microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction has been described in association with sirolimus and tacrolimus use. In common with all other immunosuppressants, it may increase the risk of opportunistic infections and post-transplant lymphoproliferative disorders.

11/18/04

14.3 Pharmacology Description

Similar to the calcineurin inhibitors, sirolimus has low oral bioavailability due to first pass metabolism by the liver and intestinal wall as well as poor absorption because it is countertransported in the gut lumen by the multidrug efflux pump, P-glycoprotein (P-gp). However, the median t_{max} value for the oral suspension is <1 hour indicating that there is rapid absorption, however, systemic availability is ~14% in stable renal transplant patients. Total body clearance is 127 to 240 mL/hr/kg and is not related to dose. Half-life is 57 to 63 hours. It is extensively bound by blood cells and plasma proteins. It is excreted by hepatic and gut metabolism. In phase III studies, whole blood trough levels for a 2 mg/day dose were 8.59 ± 4.01 ng/mL and correlate with the AUC (r^2=0.95). Only ~2% is excreted in the urine.

The mean bioavailability of sirolimus after administration of the tablet is about 27% higher relative to the oral solution. Sirolimus oral tablets are not bioequivalent to the oral solution; however, clinical equivalence has been demonstrated at the 2-mg dose level. Differences in absorption kinetics and bioavailability in renal transplant patients are shown in the table below:

Formulation, 2 mg	$C_{max,ss}$ (ng/mL)	t_{max} (h)	AUC $_{0\rightarrow\infty}$ (ng•h/mL)	CL/F/WT (mL/h/kg)
Oral solution	14.4 ± 5.3	2.12 ± 0.84	194 ± 78	173 ± 50

Tablet	15.0 ± 4.9	3.46 ± 2.40	230 ± 67	139 ± 63

Renal failure does not affect the excretion of sirolimus, but excretion is reduced in liver failure as shown below:

Population	$C_{max,ss}$ (ng/mL)	t_{max} (h)	AUC $_{0\rightarrow\infty}$ (ng•h/mL)	CL/F/WT (mL/h/kg)
Healthy subjects	78.2 ± 18.3	0.83 ± 0.17	970 ± 272	215 ± 76
Hepatic impairment	77.9 ± 23.1	0.84 ± 0.17	1567 ± 616	4 ± 62

14.5 Packaging, Formulation, Preparation and Storage

14.5.1 Sirolimus oral solution: Rapamune oral solution (bottles and foil pouches) should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). Rapamune oral solution is stable for 24 months under this storage condition. Once the bottle is opened, it should be kept in a refrigerator and the contents used within one month. However, both the opened bottles and pouches may be stored at room temperature (15°C to 30°C; 59°F to 86°F) for up to one month. An amber syringe and cap are provided for dosing from the bottle and the product may be kept in the syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). The syringe should be discarded after one use. After dilution, the preparation should be used immediately. Rapamune oral solution is provided in bottles may develop a slight haze when refrigerated. If such a haze occurs allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product.

14.5.2 Sirolimus tablets: Rapamune tablets are available as white triangular shaped tablets marked “RAPAMUNE 1 mg” in bottles of 100 tablets or as cartons containing ten blister cards of ten tablets each. Rapamune tablets should be stored at 20° to 25°C (68°-77°F). Cartons should be used to protect the blister cards and strips from light. Rapamune tablets should be dispensed in a tight, light-resistant container.

14.5.3 Supplies: Commercially available.

APPENDIX B

Methotrexate, Cyclosporine and MMF Dose Modification Guidelines

Methotrexate Dose Modifications

Guidelines for methotrexate dose adjustments for the indicated toxicities are provided in the following table. Refer to Appendix 14.4 for toxicity grading criteria.

	Grade I Mild	Grade II Moderate	Grade III Severe	Grade IV Life-threatening
	% Methotrexate Dose Reduction			
RENAL				
Creatinine	0	50	100	100
GI				
Mucositis	0	0	50	100
Stomatitis	0	0	50	100
Esophagitis	0	0	50	100
Dysphagia	0	0	50	100
CARDIAC				
Peripheral Capillary Leak				
Day 1-3	0	0	50	
Day 6-11	0	0	100	100

Routine leucovorin rescue is not allowed. Guidelines for MTX dose adjustment in patients with renal dysfunction, mucositis, or peripheral capillary leak are outlined in Appendix 14.3.1. In such patients, MTX levels may be checked beginning 24 hours after MTX administration and if the MTX level is high, leucovorin may be administered according to institutional standards/guidelines.

Cyclosporine Dose Modfication Guidelines

Cyclosporine trough levels should be measured weekly during the first 50 days post-transplant. With a fluorescence polarization immunoassay (TDX), plasma cyclosporine levels between 200-600 ng/mL (170-500 by HPLC; whole blood levels by TDX of 900-1500 ng/mL) are acceptable in patients who manifest no evidence of toxicity or GVHD.

It is essential that plasma cyclosporine levels represent trough values. Trough plasma cyclosporine levels should be drawn 10-12 hours after the last dose. When cyclosporine is being administered by 24-hour

continuous infusion, the levels can be drawn at any time. Cyclosporine levels should not be drawn from the catheter lumen used for the intravenous infusion of cyclosporine.

Adjustment of cyclosporine dose should not be based exclusively on plasma levels. Studies have demonstrated only a weak relationship between cyclosporine levels (plasma serum or whole blood) and the occurrence of GVHD or solid organ graft rejection. A better but still weak relationship between cyclosporine levels and toxicity has been demonstrated. Other factors may be important in determining clinical outcome. Biologic availability of the drug to the cells may be determined by the serum lipid concentration and profile (HDL, LDL, VLDL). Cellular factors (cyclophilin, calmodulin, calcineurin) determining the biologic effectiveness of cyclosporine may also vary between patients. Therefore, cyclosporine levels should be used as a guide in conjunction with clinical observations of the biologic effects of the drug, i.e., toxicity and immunosuppression. Guidelines provided in the following table are based on renal or neurologic toxicities as measured by creatinine (renal); neurosensory, neuro-motor, neuro-cortical, neuro-cerebellar, neuro-mood and neurovision (neurological) parameters.

Plasma Levels TDX	Plasma Levels HPLC	Toxicity Grade	Cyclosporine Dose Modification
< 200 ng/Ml	< 170	0	Increase 25%
201-600 ng/mL	171-500	0- I	No change
201-600 ng/mL	171-500	II	Decrease 50%
201-600 ng/mL	171-500	III-IV	Decrease 100%
601 ng/mL	>501	0	Decrease 25% every 3-4 days

Guidelines for MMF Dose Adjustment

If in clinical judgment of the investigator the observed toxicity is related to MMF administration, a dose adjustment will occur. Based on previous organ transplant studies, dose adjustment may occur because of gastrointestinal adverse effects. If G.I. toxicity requires medical intervention including medication for control of persistent vomiting or diarrhea and is considered not related to GVHD, a dose reduction to 50% will occur first and if there is no improvement, MMF will be stopped. For more severe G.I. toxicity, MMF will be stopped. Patients should be evaluated by the primary investigator or the GVHD attending physician before reducing MMF. A consult with Gastroenterology is also recommended if the study drug is to be stopped. If renal insufficiency develops with an estimated creatinine clearance of 25-60 ml/mm, the dose

of MMF will be reduced to 75% of the calculated dose by ideal body weight of the patient. If the estimated creatinine clearance is <25 ml, the dose of MMF will be reduced to 50%. No adjustments of MMF are required for liver dysfunction.

APPENDIX C

FRACTIONATED TOTAL BODY IRRADIATION SCHEMA

Session	Day	Time	Direction	Energy	Dose (cGy)	notes
1	-7	7:30	AP	8X or 10X	120 cGy	TLD, ck testes thickness (ALL only)
2	-7	12	PA	8X or 10X	120 cGy	TLD
3	-7	16:30	AP	8X or 10X	120 cGy	
4	-6	7:30	PA	8X or 10X	120 cGy	
5	-6	12	AP	8X or 10X	120 cGy	
			AP rt & lt CW	electrons BOOST	300 cGy	to pleural surface
6	-6	16:30	PA	8X or 10X	120 cGy	
			PA rt & lt CW	electrons BOOST	300 cGy	to pleural surface
7	-5	7:30	AP	8X or 10X	120 cGy	
8	-5	12	PA	8X or 10X	120 cGy	
			AP rt & lt CW	electrons BOOST	300 cGy	to pleural surface
9	-5	16:30	AP	8X or 10X	120 cGy	
			PA rt & lt CW	electrons BOOST	300 cGy	to pleural surface
10	-4	7:30	PA	8X or 10X	120 cGy	
11	-4	12	AP	8X or 10X	120 cGy	

Photon dose is calculated at mid-depth, central axis. Electron energies are selected as per computerized treatment planning system to deliver 300 cGy/fx to the pleural surface of each chest wall field. AP and PA portal films will be obtained prior to treatment to assure proper lung block placement.

During the first AP and PA sessions, the radiation dose received by the patient will be monitored by means of thermoluminescent dosimeters (TLD) placed on the anterior surface of the patient's skin at the following anatomic locations: forehead, SSN, Xiphoid process, umbilicus, pelvis, mid-thigh, knee, mid-calf, and ankle. The middle dose to the patient can be calculated from the sum of entrance and exit doses recorded by TLD dosimeter. If the dosimeter indicates a deviation of greater than $\pm$ 5% ($\pm$ 10% for lower extremities) in the planned dose, then either the compensating filter, the machine monitor units (due to change in patient's thickness) , or both may be modified for subsequent sessions.

APPENDIX D

GVHD ASSESSMENT

ASSESSMENT OF ACUTE GVHD

CRITERIA FOR ASSESSMENT OF GVHD

SKIN

Stage

+1 A maculopapular eruption involving less than 25% of the body surface.
+2 A maculopapular eruption involving 25-50% of the body surface.
+3 Generalized erythroderma.
+4 Generalized erythroderma with bullous formation and often with desquamation.

LIVER

Stage

+1 Moderate increase of SGOT (150-750 IU) and bilirubin (2.0-2.9 mg/dl).
+2 Bilirubin rise (3.0-5.9 mg/dl) with or without an increase in SGOT.
+3 Bilirubin rise (6.0-14.9 mg/dl) with or without an increase in SGOT.
+4 Bilirubin rise to >15 mg/dl with or without an increase in SGOT.

GUT

Stage

+1 >30 ml/kg or >500 ml of stool/day or biopsy for GVHD.
+2 >60 ml/kg or >1,000 ml of stool/day.
+3 >90 ml/kg or 1,500 ml stool/day.
+4 >90 ml/kg with abdominal pain or >2,000 ml of stool/day.

Diarrhea volume is measured as the average for the day of evaluation and the two preceding days, in order to minimize errors caused by large day-to-day variation.

Reference: Modified from Glucksberg, et al., Transplantation. 1974; 18/295-304.

OVERALL STAGING - GVHD

Degree of Organ Involvement	Grade
Grade 1-2 skin rash with no GI or liver involvement and no clinical impairment.	I (mild)
Grade 1-3 skin rash with grade 1 GI or liver involvement or both, and mild clinical impairment or grade 3 skin only.	II (moderate)
Grade 2-3 skin rash with grade 2-3 GI or liver involvement or both, and marked clinical impairment.	III (severe)
Grade 2-4 skin rash with grade 2-4 GI or liver involvement or both, and extreme clinical impairment, or grade 4 skin only with desquamation and extreme clinical impairment.	IV (life-threatening)

CHRONIC GVHD GRADING SCALE

Limited Chronic GVHD

Either or both:

1. Localized skin involvement
2. Hepatic dysfunction

Extensive Chronic GVHD

Either:

1. Generalized skin involvement, or
2. Localized skin involvement and/or hepatic dysfunction, plus
    a. Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis, or
    b. Involvement of eye: Schirmer's test with <5 mm wetting, or
    c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy specimen, or
    d. Involvement of any other target organ (e.g., esophageal abnormalities, polymyositis)

Sullivan K. Acute and chronic Graft-versus-Host Disease in Man. Int J Cell Cloning. 1986;4:42-93 (suppl 1).

APPENDIX E

PERFORMANCE STATUS

%	Karnofsky†	Grade	WHO*	Status	ECOG**
100	Normal; no complaints/ no evidence of disease	0	Able to carry out normal activity without restriction	0	Normal activity
90	Able to carry on normal activity; minor signs or symptoms of disease				
		1	Restricted in physically strenuous activity but ambulatory and able to carry out work	1	Symptoms but ambulatory
80	Normal activity with effort; some signs or symptoms of disease				
70	Cares for self; unable to carry on normal activity or do active work				
		2	Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours	2	In bed <50% of time
60	Requires occasional assistance but is able to care for most of his needs				
50	Requires considerable assistance and frequent medical care				
		3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours	3	In bed >50% of time
40	Disabled, requires special care and assistance				
30	Severely disabled; hospitalization is indicated though death not imminent				
		4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair	4	100% bedridden
20	Very sick; hospitalization is necessary				
10	Moribund; fatal process progressing rapidly				

* WHO = World Health Organization ** ECOG = Eastern Cooperative Oncology Group
† Karnofsky= D.A., et al., Cancer 1: 634-656, 1948

APPENDIX F

STUDY CALENDAR

TESTING	PRE-BMT	WEEK 1	WEEK 2	WEEK 3	WEEK 4/ DAY 30	DAY 60	DAY 100	6 MONTHS	1 YEAR
HISTORY AND PHYSICAL EXAM	X				X	X	X	X	X
KPS	X				X	X	X	X	X
LABORATORY:									
CBC/PLATELETS	X	Qd	qd	qd	qd	X	X	X	X
SMA-18 + Mg	X	q M-W-F	q M-W-F	q M-W-F	q M-W-F	X	X	X	X
CMV Blood Cultures*	X			q Mon + Thurs	q Mon + Thurs until day 100				
Ig Levels	X	X	X	X	X	X	X	X	X
24 hour Urine Clearance	X								
HIV, Hepatitis Screen	X								
CMV Titer (Serum)	X								
HSV Titer	X								
OTHER TESTS:									
CXR	X	X	X	X	X				
Cardiac Echo	X								
Chest/Abd CT	X								
BM Asp + Bx †	X				X		X	X	X
Chimerism Study					X	X	X	X	X
GVHD ASSESSMENT					X	X	X	X	X
TOXICITY ASSESSMENT					X	X	X	X	X

*** CMV Bl C/S or PCR analysis**
† For patients with bone marrow involvement at transplant

8/6/02

APPENDIX G

IV BUSULFAN KINETICS FORM

IRB#: _____ DATE: _____ (CIRCLE WEIGHT USED)

PATIENT _____ DOSE #_____ Actual Body Weight _____

Medical Record #_____ Start time: _____ Ideal Body Weight _____

MD/Pager # _____ Stop Time: _____ Adjusted IBW _____

Coordinator/Pager #_____ DOSE: _____mg (_____mg/m^2) BSA_____m^2

INFUSION TO RUN OVER 2 HOURS

****LABEL EACH TUBE BY NUMBER CONSECUTIVELY WITH THE APPROPRIATE BLOOD DRAW TIME**

Tube #	Collection Schedule	Proposed Collection Time	Actual Collection Time
1	Immediately prior to beginning of infusion	APPROX. 0555	
2	Immediately prior to end of infusion	APPROX. 0755	
3	15 minutes post infusion	APPROX. 0815	
4	30 minutes post infusion	APPROX. 0830	
5	60 minutes post infusion (1 hour)	APPROX. 0900	
6	180 minutes post infusion (3 hours)	APPROX. 1100	
7	240 minutes post infusion (4 hours)	APPROX. 1200	

CALL SPD FOR STAT PICK-UP AFTER EACH BLOOD DRAW

CALL Cara Ortega at 62681 or Jim Manwaring at 64515 if not picked up within 15 minutes

1. ALL samples to be obtained in 7cc green top tubes (Sodium Heparin). DO NOT draw from Busulfan line. (use opposite port of Hickman Catheter for blood draw).
2. After EACH DRAW, send sample on ice to HLA LAB via SPD.
3. After last draw, call SPD STAT for pick-up to take to HLA LAB.
4. Send a copy of this completed form to HLA LAB with last blood draw.

Please call, ext 62394 or Kathy Patane, ext 63238 to pick up this form.

Addressograph

RN SIGNATURE _____