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DEPARTMENT OF MEDICAL ONCOLOGY AND THERAPEUTICS RESEARCH

TITLE: Sequential High-Dose Melphalan and Busulfan/Cyclophosphamide Followed by Peripheral Blood Progenitor Cell Rescue, Interferon, Pamidronate, With or Without Thalidomide, for Patients With Multiple Myeloma

CITY OF HOPE PROTOCOL NUMBER/VERSION: **IRB # 99021** **VERSION: 09**

DATE(S)/ OF AMENDMENT(S)/REVISION(S): **Version:**

C.O.H. Amendment #1	06/14/99	01
C.O.H. Amendment #2	08/12/99	02
C.O.H. Amendment #3	09/27/99	03
C.O.H. Amendment #4	01/07/00	04
C.O.H. Amendment #5	03/08/00	05
C.O.H. Amendment (consent only)	11/17/00	00
C.O.H. Amendment #6	02/22/01	06
C.O.H. Amendment #7	03/13/02	07
C.O.H. Amendment #8	10/09/02	08
C.O.H. Amendment #9	02/03/04	09

SITE: Bone Marrow

HISTOLOGY: Myeloma

STAGE (If applicable): I-III

MODALITY: High-Dose Chemotherapy and Stem Cell Support / Immunotherapy

TYPE: Phase II

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01/07/00

SCHEMA

PRIMING and APHERESIS — CYCLOPHOSPHAMIDE 1.5 GM/M^2 and FILGRASTIM 10 µg/kg/Day

CYCLE 1

DAY -1 MELPHALAN 150 MG/M^2 IV

DAY 0 REINFUSION OF COLLECTED STEM CELLS

DAY 1 START FILGRASTIM 5 MICROGRAMS/KG, DAILY, IV

CYCLE 2 TO START AT LEAST 12 WEEKS FROM DAY 1 and NOT BEYOND 18 WEEKS

DAY -8 START DILANTIN
DAY -7 BUSULFAN 0.8 MG/KG EVERY 6 HR x 4 IV
-6 BUSULFAN 0.8 MG/KG EVERY 6 HR x 4 IV
-5 BUSULFAN 0.8 MG/KG EVERY 6 HR x 4 IV
-4 BUSULFAN 0.8 MG/KG EVERY 6 HR x 4 IV

DAY -3 CYCLOPHOSPHAMIDE 60 MG/KG; START MESNA IV
-2 CYCLOPHOSPHAMIDE 60 MG/KG; CONTINUE WITH MESNA IV

03/08/00 DAY 0 REINFUSION OF COLLECTED STEM CELLS (PBPC) - START FILGRASTIM 5 MICROGRAM/KG, DAILY

10/10/02 STARTING WITHIN 12-18 WEEKS OF DAY ZERO OF CYCLE 2, ALPHA INTERFERON 3 x 10^6 UNIT/M^2 TIW FOR 3 YEARS AND AREDIA 90 MG IV. Q 4 WEEKS

AT 6 MONTHS FOLLOWING DAY ZERO OF CYCLE 2, THALIDOMIDE 400 MG/M^2 WILL BE ADMINISTERED ORALLY TO PATIENTS WHO WILL NOT HAVE ACHIEVED COMPLETE REMISSION BY THAT TIME

1.0 OBJECTIVES

01/07/00 1.1 PRIMARY OBJECTIVES

1.1.1 To assess the feasibility and toxicities of tandem cycle high-dose melphalan and busulfan/cyclophosphamide followed by autologous peripheral blood progenitor cell rescue (PBPC), alpha-interferon and pamidronate in patients with responsive, or stable "low bulk" multiple myeloma.

1.1.2 To assess response rate, progression-free and overall survival following treatment with tandem cycle, high-dose melphalan, busulfan/cyclophos-phamide, PBPC and maintenance therapy with alpha-interferon and pamidronate in patients with responsive, or stable "low bulk" multiple myeloma.

1.1.3 To assess the feasibility of adding thalidomide to alpha-interferon and pamidronate in patients not in complete remission by 6 months following the second cycle of high-dose therapy.

1.1.4 To assess whether administration of thalidomide can increase the complete remission rate (CR) in patients not in CR at 6 months after the second cycle of high-dose therapy and evaluate its effect on progression-free and overall survival in this subset of patients.

1.2 SECONDARY OBJECTIVES

1.2.1 To perform cytogenetic, gene rearrangement and fluorescence *in situ* hybridization (FISH) studies on baseline and post-treatment bone marrow and blood specimens and correlate the presence/persistence of these features with treatment outcome.

02/22/01 1.2.2 To monitor the pharmacokinetics of busulfan and assess any correlation with toxicities and outcome in the subject population.

1.2.3 To assess the effect of thalidomide on microvascular density of bone marrow and correlation of such possible effect on outcome.

01/07/00 2.0 BACKGROUND AND RATIONALE

2.1 The incidence of multiple myeloma (MM) has been on the rise accounting for approximately 10% of all hematological malignancies. Five in 100,000 Americans suffer from MM; the entire spectrum of plasma cell dyscrasias affects an even greater percentage of the population. These diseases include benign gammopathy, macroglobulinemia, solitary plasmacytoma and, within the category of MM, smoldering and indolent forms as well as immunoblastic lymphoma and plasma cell leukemia[1]. Adverse prognostic factors such as high plasma cell labeling index, elevated beta-2 microglobulin and C-reactive protein, the presence of specific chromosomal abnormalities, plasmablastic morphology and abnormal renal function, poor performance status at diagnosis and elevated serum levels of interleukin-6 are of grave clinical significance.[2-4] Chemotherapy with melphalan and prednisone, a combination of alkylating agents, or vincristine, doxorubicin is effective in 50-70% of patients with newly diagnosed MM; however, less than 10% of patients will achieve complete remission[5,6].

Unfortunately, the effects of chemotherapy are usually short-lasting. The median survival of patients with MM is 30-36 months. The lack of long-term effectiveness is due to either primary, or acquired resistance. Attempts to improve overall response and response duration have been focusing on overcoming drug resistance due to overexpression of the MDR-1 gene product[7], or utilizing myeloablative therapy followed by allogeneic, or autologous bone marrow, or peripheral blood progenitor cell rescue (PBPC).[8]

2.2 High-dose chemo-/radiotherapy in the treatment of MM.

Single agent high-dose melphalan therapy results in response rates of > 80% with complete response rates of > 30% as described in earlier studies without the use of stem cell support. In a recent update, 1/3 of the patients from the original cohort treated with high-dose melphalan were reported to be alive at 9 years[9,10]. Thousands of patients with multiple myeloma have received dose-intense bone marrow ablative therapy followed by autologous bone marrow, or peripheral stem cell rescue, worldwide. Complete remission rates of up to 50% have been reported in selected cohorts of patients with good performance status and limited tumor burden resulting from a response to conventional doses of chemotherapy.[8] Induction regimens consisting of total body radiation with cyclophosphamide or melphalan, or combination chemotherapy with and cyclophosphamide have resulted in similar outcome in a variety of phase II trials.[11-16] A prospective randomized study comparing high-dose consolidation therapy and stem cell rescue following induction therapy vs. conventional chemotherapy alone, revealed complete response rates (22% versus 5%) and improved favorable progression-free and overall survivals following high-dose therapy; projected 5 year event-free and overall survivals were 28% and 52% versus 10% and 12%.[17] Comparison of early vs. late (at the time of progression) autologous transplants favor early high-dose therapy resulting in prolonged progression-free survival and quality of life while in response.[18] Tandem cycle high-dose chemo/radiotherapy in the setting of "total therapy" in the largest series by a single institution suggested additional benefit associated with the second transplant in a series of 231 patients, independent of the presence of unfavorable cytogenetics and elevated beta 2 microglobulin; with a median follow-up of 4.2 years among surviving patients actuarial 5-year event-free and overall survivals were 58% and 42%, respectively.[19] The benefits of double dose intense therapy need further confirmation in ongoing randomized prospective trials. Preliminary data from one such French study suggests a possible benefit favoring double transplant; similarly, EBMT data and preliminary analysis of an Italian randomized study comparing single versus tandem autologous transplant favors tandem therapy.[20-22]

Allogeneic bone marrow transplantation has been associated with a disappointing median overall survival of 13 months in a registry review by the European Group for Blood and Bone Marrow Transplantation, with approximately 40% early, treatment-related mortality caused by regimen-related toxicities, infections, and acute or chronic graft versus host disease.[16] More recent data suggest diminished early mortality rates possible due to incorpotation of PBPC reinfusions in addition to ongoing assessment of the role of the so called mini transplants.[23,24, 25,]

Early utilization of myeloablative therapy is recommended both with autologous and allogeneic bone marrow transplantation in order to consolidate complete or partial response to conventional treatment. In addition, even patients with previously untreated or refractory disease may respond to high-dose therapy, with the possibility of long-term disease control.[8]

2.3 Autologous $CD34^+$ selected PBPC support following high-dose therapy in MM.

Occult tumor involvement is one of the potential risks associated with the use of a autologous stem cell products. The percentage of myeloma cells contaminating the PBPC product and bone marrow were 0.0024% versus 0.74% using patient-specific oligonucleotide primers synthesized to identify patient specific Ig gene rearrangements and than amplified by PCR; these data suggest a potential benefit when using PBPC.[26] Methods of $CD34^+$ selection for tumor purging might benefit patients without significant delay in hematopoietic recovery. However, the clonogenic potential and contribution to disease progression of MM cells residing in the PBPC products in relation to the body's total tumor burden is unknown. Indeed, more mature data comparing outcome in patients supported by CD34+ selected versus standard PBPC following high-dose therapy for MM was not supporting the use of such technology in patients with advanced disease.[27] In this study only *in vivo* "purging" with cyclophosphamide followed by Filgrastim will be utilized in the setting of PBPC mobilization and processing.

2.4 Interferon maintenance

Alpha interferon has been found to exhibit antitumor activity in the therapy of multiple myeloma. Maintenance therapy following both chemotherapy and after bone marrow transplantation seems to contribute toward prolonged disease-free and overall survival.[28]

2.5 Pamidronate in the therapy of MM

Bisphosphonates –presumably by interfering with osteoclast-mediated bone resorption- are useful in reducing the incidence/complications of lytic metastases. The bisphosphonate pamidronate, in a randomized, prospective study had been found effective in reducing the number skeletal complications; pamidronate may also alter the natural history of MM by directly interfering with the degree of plasmocytosis.[29,30]

2.6 Thalidomide in the therapy of MM

In bone marrow samples of patients with active MM there is ample evidence for active angiogenesis;[31] expression of certain adhesion molecules is also increased on the surface of such plasma cells in comparison to those from patients with MGUS.[32] In at least one study diminished vascularity following high-dose therapy and PBPC seemed to be associated with better outcome.

Both interferon alpha-2 and pamidronate have the potential to inhibit angiogenesis through interfering with the effects of IL-6, TGF beta and other, so far undefined pathways, leading to prolonged survival.[28],[34] More recently, thalidomide, and agent with possibly antiangiogenesis potential had been reported to induce tumor response-including short-lasting complete remissions- in heavily treated, relapsed patients with MM.[35]

2.7 Chromosomal abnormalities as prognostic indicators in MM

Cytogenetic abnormalities can be detected in significant numbers of MM cases; the presence of 11q, -13 and deletion 13q have been associated with worse survival;[19] frequent abnormalities observed in MM include t(4;14) (p16;q32), t(11;14) (q13;q32), t(8;14) (q24;32), t(14;18) 9 q32;q21), 13q14 (Rb loss) and others. [36] One of these translocations, t(4;14) may lead to dysregulations of two separate oncogenes providing one of many potential targets for specific interventions in the future.[37]

Autologous stem cell transplantation following high-dose chemo-/radiotherapy may contribute to the development of clonal hematopoietic evolution (or unmask/accelerate previously existing clonal abnormalities). Hence, we will apply a unique assay (specific only in female patients) to detect the presence of clonal hematopoiesis prior to high-dose therapy as well as to monitor for the development of such clones following treatment, in female participants.

2.8 Cytogenetic and fluorescence *in situ* hybridization (fish) studies in multiple myeloma

Classic cytogenetics and FISH analyses will be used to detect clonal aberrations in multiple myeloma. Clonal aberrations will be used to monitor therapy and detect minimal residual disease levels at specified time intervals. Classic cytogenetics will be performed on the baseline (pretreatment) bone marrow study using standard methods. In addition, a unique system, which utilizes FISH for the simultaneous detection of multiple chromosome aberrations on a single slide with labeled chromosome probes, will be used at five-scheduled time points. The Multiprobe is a disposable glass device consisting of a 3 x 8 array of raised bosses; each with different chromosome specific probes reversibly bound to them.[38]

In this study, two slides will be used per scheduled time point. One slide will be use the Chromoprobe_ Multiprobe I System for the rapid evaluation of cell ploidy and to determine the chromosomal origin of small marker chromosomes. The probes used are pericentromeric repeat sequences and satellite probes; this is often the only portion of the original chromosome constituting the marker. Using this technique, we will test for gains or losses of whole chromosomes with 8 to 10 different chromosome specific probes (1, 3, 6, 7, 9, 11, 17, and 18) to identify and further characterize aneuploidy in patients with multiple myeloma. The second slide has been customized to identify myeloma-specific cytogenetic aberrations. Four translocations affecting 14q32 and two locus-specific deletions will be studied. These non-random aberrations have been determined to be the most common in multiple myeloma, namely, t(4;14)(p16;q32), t(11;14)(q13;q32), t(8;14)(q24;q32), t(14;18)9q32;q21), 13q14 (Rb loss) with 13q telomere probe and TP53 loss with a chromosome 17 telomere probe.[37] Multiprobe FISH complements conventional cytogenetics and can be used to screen normal, failed and poorly described cases of multiple myeloma. Use of this approach will increase the identification of patients with multiple

myeloma tumor markers and enable them to be assigned to prognostically important subgroups, as reported for acute lymphoblastic leukemia.[39]

2.9 Humara assay methodology

To determine whether dose-intensive adjuvant regimens for multiple myeloma induce genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis, female patients will be monitored for clonality by the HUMARA. The HUMARA assay is a PCR-based test for detecting clonality utilizing the human androgen receptor locus on the X chromosome. The assay takes advantage of dosage compensation in humans achieved through random inactivation of one of the two X chromosomes in the cells of normal females. This inactivation occurs early in development and thus females are essentially cellular mosaics for the genes on the X chromosome, that is, some cells have either the maternal or paternal X chromosome inactivated. This genetic concept is also known as the Lyon hypothesis. In theory, all normal female tissue would be randomly methylated with 50% of cells having the paternal (pat) allele inactivated and 50% of the cells having the maternal (mat) allele inactivated; the ratio of relative methylation would be 50:50, or a ratio of 1. This pattern of inactivation is maintained faithfully in all progeny.[40]

Clonality at the HUMARA locus is assessed by nested PCR amplification, according to Mach-Pascual *et al*.[41] and quantitated by the method of Delabesse *et al*.[42] The HUMARA assay is designed to amplify a ~250 to 300 base pair (bp) region of the first exon of the human androgen receptor. Two *Hpa II* methylation sensitive sites reside within 100 bp 5' to the polymorphic CAG repeat. Primers flank the methylation sensitive restriction enzyme sites and the CAG repeat simultaneously. Methylated enzyme (*Hpa II*) sites correlate with X inactivation. Unmethylated alleles (active X) are digested by *Hpa II* and eliminated from PCR amplification. The methylated or inactive allele will remain intact after the *Hpa II* digestion and is the only allele amplified. After amplification, the maternal and paternal alleles are resolved using a sequencing gel. Random inactivation will show both maternal and paternal alleles, signifying a polyclonal state; whereas a clonal population will be identified by the presence of one allele or a shift of greater than 3-fold, to control for skewed X-inactivation over the other allele.
The allele ratio is defined as the ratio between the two chromosome X-linked alleles in a given sample. The corrected ratio is determined by dividing *Rsa* I-*Hpa* II by *Rsa* I alone. This corrects for preferential amplification of one of the two alleles. Those samples with a corrected ratio of less than 3 are considered within normal limits. Those with a corrected ratio greater than 3 are consistent with either skewed X inactivation or clonal hematopoiesis. Although criteria for nonrandom X-inactivation are arbitrary, an allele ratio $\geq$ 3:1, which corresponds to the expression of 75% of one allele, has been widely accepted in the literature.[42, 43] To normalize for excessive skewing, which occurs in ~20% of the female population and appears to increase with aging,[44] the ratio of the two alleles in the experimental tissue (polymorphonuclear cells) must be divided by the ratio of the same two alleles in normal somatic control tissue (T-cells). If the ratio remains greater than 3, the results are consistent with clonal hematopoiesis.

2.10 COHNMC experience with tandem cycle high-dose therapy in MM

Between 12/94 and 1/99, 44 patients with multiple myeloma have been enrolled on our prior tandem cycle high-dose chemotherapy trial. Patients ≤ 65 years, with a performance status of >70%, creatinine clearance of >70 cc/min, and with ≤ 10% bone marrow involvement in response, or with stable disease were eligible. The median age was 51 (range,38-65), 23 (52%), 16 (37%), and 5 (11%) patients received 1, 2, and 3 prior chemotherapy regimens; Forty three percent of patients received prior radiation therapy; median time from diagnosis to high-dose therapy (HDCT) was 9.1 months (range,4.8-73.4). Three of forty four patients were in complete remission pre-high-dose threapy. All patients underwent PBPC priming with Filgrastim 5 μg/kg bid, sc to procure > 4 x 10^6 CD34+ cells/kg and /or > 14 x 10^6 mononuclear cells /kg. HDCT consisted of melphalan 150 mg/m2 (C1) followed by busulfan 4 mg/kg/day x 4 and cyclophosphamide 60 mg/kg/day x 2 days (C2); each cycle was followed by PBPC. Alpha interferon 3 x 10^6 units /m^2 three times a week, sc was then started for 2 years. Four of 31 patients (9%) did not receive C2 due to progression (n=1) deterioration in renal function (n=1), dental abscess delaying admission (n=1) or psychosocial problems (n=1). C2 followed C1 in 2.1 months (range, 0.9-3.9). Two patients died of hepato-renal failure (VOD), 4 pts with VOD recovered but one of them subsequently died of systemic candidiasis. Busulfan pharmacokinetics were measured in 23 of the first 27 pts treated with both C-s (85%); the median area under the curve (AUC) of busulfan in patients developing VOD (1150 uM*min, range, 788-1323) was not higher then in pts without VOD (1128 uM*min, range, 622-1537). The number of days to absolute granulocyte recovery after C1 was 11.5 and after C 2 was 10, respectively. Patients became platelet independent by day 11.5 following C 1 and day 12 (7-380+) after C2; 1 patient, whose myeloid lineage recovered but never reached platelet independence suffered secondary graft failure and subsequently became transfusion-independent 11 months post C2. Forty five percent of patients in partial response pre-HDCT converted into complete remission post-HDCT. Complete remissions post-high-dose therapy were documented in ~ half of patients within 2 months following C2 and in the majority of them by 6 months. The 3 year progression-free survival is 0.54 and overall survival is 0.84 at a median post HDCT follow-up for live patients of 24.0 months (manuscript in preparation).[45]

Based on the feasibility and possibly better CR rate and progression-free survival of tandem cycles of melphalan and busulfan/cyclophosphamide followed by alpha interferon and in view of the encouraging, survival data in this relatively "low tumor bulk" population with responding, or stable multiple myeloma we propose to treat a larger cohort of patients with exposure to ≤ 3 prior treatment regimens with the same tandem regimen but with some modifications:

Patients will be eligible with up to ≤ 40% marrow involvement provided that they are either responding to induction therapy or have stable disease.

We will incorporate cyclophosphamide both as a priming agent and to provide *in vivo* purging prior to Filgrastim priming.

Tandem cycles of high-dose melphalan and then busulfan/cyclophosphamide will be administered but the dose of busulfan will be replaced with the IV preparation at 0.8 mg/kg x 16 doses in an attempt to reduce the incidence of VOD which may be caused not just due to

exposure to busulfan but possibly due to interaction between busulfan and cyclophosphamide metabolism/metabolites.

02/22/01 Pharmacokinetic measurements of busulfan will be carried out to correlate toxicities and outcome with drug kinetics.

We will prolong administration of alpha interferon for 3 years in view of preliminary data in our current cohort suggesting a "suppressive" effect of interferon on the degree of myeloma protein production (manuscript in preparation).

Pamidronate will be administered until progression at a dose of 90 mg every 4 weeks to all patients.

For patients not in complete remission by 6 months (the majority of our patients in the previous trial achieved a CR by this landmark on, or off interferon and pamidronate) thalidomide 400 mg/day will be prescribed for a period of 3-6 months in order to convert their disease into complete remission.

Cytogenetic abnormalities by standard and FISH analysis to assess their role as predictors of response, monitors of residual disease and predictors of survival will be assessed. In female patients the presence/evolution of clonal hematopoiesis will be monitored.

3.0 THERAPEUTIC AGENTS

3.1. Busulfan

3.1.1. Therapeutic classification – Alkylating agent

3.1.2 Mechanism of action – Interfere with DNA replication and transcription of RNA and ultimately results in the disruption of nucleic acid function.

3.1.3 Animal tumor data – Busulfan has been shown to be active against a variety of animal neoplasm in vivo, including mouse sarcoma 180 and Ehrlich mouse ascites tumor.

3.1.4 Animal toxicology – Busulfan fed to rats in an amount equivalent to about 0.5 mg/kg of final body weight per day slowed weight gain and produced bone marrow depression, pancytopenia and cataracts after about 10 weeks. In rats, LD50 was found to be 34 mg/kg intraperitoneally. When the drug was administered on day 13, 14, or 15 of gestation at a dose of 10 mg/kg to rats, the progeny were prematurely sterile.

3.1.5 Human pharmacology – limited pharmacology data are available for the parenteral formulation to be used in this study and is given in above. The pharmacokinetic data suggests that the plasma decay of the formulation fits a two compartment model. Busulfan is reported to be extensively metabolized, 12 metabolites have been isolated, but most have been identified. The drug is slowly excreted in the urine, chiefly as methanesulfonic acid. Ten to 50% of a dose is excreted as metabolites within 24 hours.

3.1.5.1 Example: a 70 kg patient who is to receive a dose of 0.8 mg/kg of busulfan (6 mg/mL) would receive (70 kg) x (0.8 mg/kg) / (6 mg/mL) = 9.33 mL of busulfan injection.

Calculate the amount of drug to be administered based on the dosage and the patient's body weight.

Prepare a solution of 0.09% Sodium Chloride Injection USP (normal saline) or 5% Dextrose Injection USP (D5W) that is 10 times the volume of the calculated busulfan dose in mL from the step above. For example, if the dose of busulfan for injection is 9.33 mL, prepare an appropriate container with (9.33 mL dose) (10) = 93.3 mL of normal saline or D5W.

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 drug flows into and through the solution. Mix by inverting the bag. DO NOT use if this solution contains visible particulates.

Place a suitable intravenous administration set into the outflow port of the bag containing the infusion solution.

3.1.6 Route of Administration: The drug will be given by slow intravenous infusion over two hours. CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. Peristaltic pumps or syringe pumps may be used with the busulfan solutions as prepared above. Prior to and following each infusion, clear the catheter line with normal saline (approximately 6 mL). DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

3.1.7 Pharmaceutical data: Busulfan injection is a sterile, pyrogen-free solution provided in a mixture of dimethylacetamide (DMA) and polyethylengegycol 400 (PEG400). It is supplied in 10 mL single use ampules at a concentration of 6 mg Busulfan per mL. Each ampule contains 60 mg of Busulfan in 3.3 mL of DMA and 6.7 mL of PEG400. When diluted in normal saline or D5W to a concentration of 0.5 mg/mL , the resulting solutions must be administered within eight (8) hours of preparation including the 2 hour infusion of the drug.

3.1.8 Stability and storage – stable at 4_C for at least twelve months. Additional stability studies are in progress. Ampules should be stored refrigerated at 2-8_C. DO NOT FREEZE. Ampules may be stored for up to 7 days at room temperature.

3.1.9 Solution preparation: prepare the busulfan solution as follows:

3.1.9.1 Use sterile, non-pyrogenic, disposable containers, syringes, needles, stopcocks, and transfer tubing, etc.

3.1.10 Human toxicology: Toxicology from busulfan includes:

3.1.10.1 Severe bone marrow hypoplasia which would be fatal without administration of

bone marrow stem cells.

3.1.10.2 Nausea and vomiting which can be decreased by the use of sedation and antiemetic.

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

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

3.1.10.5 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.

3.1.10.6 Temporary hyperpigmentation of the skin and nail be changes.

3.1.10.7 Grand mal seizures which can be prevented by the prophylactic administration of dilantin.

3.1.10.8 Edema: Seventy-nine percent (79%) of patients exhibited some form of edema, hypervolemia, or weight increase: all events were reported as mild or moderate.

3.1.11 Supplier: This drug is commercially available.

3.2. Cyclophosphamide

3.2.1 Mechanisms of Action: This drug is biotransformed principally in the liver to active alkylating metabolites which prevent cell division by cross linking strands of DNA and it also inhibits DNA synthesis.

3.2.2 Human Toxicity: Toxicity from cyclophosphamide includes bone marrow suppression which usually occurs ten to twelve days after administration, nausea and vomiting, reversible alopecia, hemorrhagic cystitis which can frequently be prevented with increased hydration and co-administration of MESNA, and sterility and decreased gonadal function. There are isolated reports of hemorrhagic colitis, oral mucosal ulceration, and jaundice.

3.2.3 Pharmaceutical Data: Formulation; Cyclophosphamide is supplied in 100 mg, 200 mg, and 500 mg vials as a white powder. The drug can re reconstituted in either normal saline or D5W.

6/14/99 3.2.4 Administration: The drug should be dissolved in about 500 cc of D5W and it is infused IV over 2 hours. An added dose of IV fluids may help prevent bladder toxicity. Cyclophosphamide 1.5 gm/m^2 (based on actual body weight) will be administered for mobilization, prior to apheresis. During cycle 2 of the treatment phase the drug will be administered on Days -3 and -2 at a dose of 60 mg/kg/day IBW. There will be no correction for extremely obese patients. It will be dosed by IBW. In order to prevent hemorrhagic cystitis, vigorous hydration and diuresis together with MESNA will be employed.

3.2.5 Supplier: This drug is commercially available for purchase by the third party.

3.3 Melphalan (L-phenylalanine mustard, L-PAM, Alkeran)

3.3.1 Mode of Action: In common with other nitrogen mustards, melphalan reacts with DNA to produce either DNA-DNA or DNA-proteins cross-linked products probably by binding at the N-7 position of guanine.

10/10/02

3.3.2 Supply, Reconstitution and Administration: Melphalan is commercially available by Burroughs Wellcome Company in sterile vials containing 50 mg lyophilized drug as the hydrochloride salt. It is formulated with 20 mg povidone per 50 mg vial. Sterile diluent is supplied which contains per 10 ml: sodium citrate 0.20 g, propylene glycol 6 ml, ethanol (95%) 0.526 ml, sterile water q.s. 10 ml. Reconstituted vials (undiluted solutions) are stable for 90 minutes. Melphalan diluted in NS to 0.1-0.45mg/ml is stable for only 60 minutes. Melphalan is unstable when diluted with NS to 2mg/ml. The rate of infusion should be 30 minutes or less.

3.3.3 Toxicity: The dose-limiting toxicity of melphalan is myelosuppression. Other toxicities after IV melphalan include mucositis, nausea, vomiting, and diarrhea. Alopecia is generally seen only with high doses associated with bone marrow transplant settings. Rarely reported reactions include pulmonary fibrosis, skin rash, vasculitis, and allergic reactions. With high dose chemotherapy, gastrointestinal toxicity becomes dose-limiting. At such high doses, elevated transaminases, syndrome of inappropriate antidiuretic hormone secretion, depression, interstitial pneumonitis, and hepatic veno-occlusive disease have been reported. Acute nonlymphocytic leukemia and myeloproliferative syndromes may occur as secondary cancers from any alkylating agent such as melphalan. Amenorrhea, permanent in many cases, have been noted when melphalan was used in premenopausal women undergoing adjuvant therapy for breast cancer. Azoospermia would be expected, but is not well documented in the literature.

3.4 Filgrastim (r-metHuG-CSF)

3.4.1 Description

Filgrastim is a human granulocyte colony stimulating factor (G-CSF), produced by recombinant DNA technology. NEUPOGEN® is the Amgen Inc. trademark for Filgrastim, recombinant methionyl human granulocyte colony stimulating factor (r-metHuG-CSF).

Approximately 6,400 patients in U.S. and international based trials have participated in clinical trials of Filgrastim to date, and the worldwide commercial populations receiving Filgrastim totaled approximately 190,000. The drug has been found to be well tolerated at dosages up to 60 µg/kg/day given IV or SC, with no toxic effects attributable to Filgrastim. A maximum tolerated dose has not yet been determined.

3.4.2 Contraindications

NEUPOGEN® is contraindicated in patients with known hypersensitivity to E. coli-derived proteins, Filgrastim, or any component of the product.

3.4.3 Adverse Reactions

The only consistently observed clinical toxicity described with Neupogen® is medullary bone pain. Other clinical toxicities that have been described include skin rash, and cutaneous vasculitis. Since commercial introduction of Neupogen®, there have been rare reports of allergic-type reactions. Biochemical abnormalities that may occur include increases in alkaline phosphatase, uric acid, and lactate deydrogenase.

3.4.4 Dilution and Storage

If required, NEUPOGEN® may be diluted in 5% dextrose. NEUPOGEN® diluted to concentrations between 5 and 15 mcg/mL should be protected from adsorption to plastic materials by addition of Albumin (Human) to a final concentration of 2 mg/mL. **Do not dilute with saline at any time; product may precipitate.**

NEUPOGEN® should be stored in the refrigerator at 2-8 degrees Centigrade (36-46 degrees Fahrenheit). Do not freeze. Avoid shaking. Prior to injection, NEUPOGEN® may be allowed to reach room temperature for a maximum of 24 hours. Any vial left at room temperature for greater than 24 hours should be discarded.

3.4.5 Supplier

Commercial NEUPOGEN® is available in 1 mL and 1.6 mL vials at a concentration of 300 mcg/mL. Discard unused portions. Use only one dose per vial; do not reenter the vial. Do not save unused drug for later administration.

3.5 MESNA (NSC-113891)

3.5.1 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 100 mg/ml solution (400 mg/4 ml ampule). It may be administered intravenously by bolus and/or continuous infusion.

Toxicity

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

3.6 Interferon-Alpha-2b

3.6.1 Mechanism of action: Alpha-2b interferon has a wide variety of antiproliferative, antiviral and immunomodulatory effects. The major mechanism of effect in multiple myeloma is

probably an antiproliferative one directly against the myeloma cells - an effect which has been documented by *in vitro* studies. However, both immunomodulatory and antiviral effects cannot be excluded in the clinical setting.

3.6.2 Human toxicity: The major toxicity of alpha-2b interferon is a "flu-like" syndrome, which occurs in a dose dependent fashion and consists primarily of fever, chills, myalgias, arthralgias and headache. At the three million unit dosage schedule the syndrome is minor or non-existent. Fatigue with decrease in performance status is also usually mild at this dosage as is the objective toxicity such as neutropenia, anemia and reversible liver transaminase elevations. Autoimmune thyroid disease has been observed in patients receiving alpha-interferon.

3.6.3 Pharmaceutical data: Alpha-2b interferon will be supplied in vials containing a sterile lyophilized powder. The 5 million and 10 million IU vials are for use by intramuscular or subcutaneous injection and will provide 5 x 10^6 IU of interferon activity when reconstituted.

3.6.4 Storage: Lyophilized Powder - Vials must be stored in a secured refrigerator at 2º - 8ºC (36º - 46ºF).

3.6.4.1 Reconstituted Solution

The reconstituted solution should be refrigerated at 2º - 8ºC (36º - 46ºF) and used within 24 hours or frozen at –20ºC.

3.6.4.2 Reconstitution

Each 5 x 10^6 IU vial requires 1.0 ml of preservative-free sterile water for reconstituting the lyophilized powder into solution for injection. Each 10 x 10^6 IU vial requires 2.0 mls of diluent. It is the responsibility of the investigator to supply the sterile water. Each vial may be used once only; any unused material will be discarded.

3.6.5 Supplier: Alpha-2b interferon is commercially available for purchase by a third party.

01/07/00 3.7 Thalidomide (NSC #66847)

3.7.1 Other Name: Thalomid™

3.7.2 Molecular Formula: $C_{13}O_4N_2H_9$ (M.W. 243)

3.7.3 Description: Thalidomide is a racemate.

3.7.4 How Supplied: Supplied by Celgene as 50 mg hard gelatin capsules

3.7.5 Storage: Thalidomide should be stored at room temperature.

3.7.6 Route of Administration: Oral

3.7.7 Toxicities: Drowsiness and sedation, headache, constipation, nausea, dryness of mucosa, erythematous skin eruptions, peripheral neuropathy, increased appetite, weight gain, loss of libido, edema of face and extremities, galactorrhea, dry skin, leukopenia, menstrual abnormalities, pruritis, alopecia, eosinophillia, somnolence, depression and of course, teratogenic effects.

3.7.8 **WARNING**: There is an extremely high risk that a deformed infant will result if pregnancy occurs while taking thalidomide even for short periods. Therefore, this teratogenic action of thalidomide necessitates:

3.7.8.1 Female patients having any chance of becoming pregnant must have a pregnancy test performed within 24 hours of beginning thalidomide, weekly for the first 4 weeks of treatment, and then evry 4 weeks if the patients periods are regular or every 2 weeks if they are not.

3.7.8.2 Female patients mus either abstain from all reproductive sexual intercourse or use 2 methods of birth control at least 1 highly active method (*e.g. intrauterine device [IUD], hormonal [birth control pills, injections, or implants], tubal ligation, or partner's vasectomy*) and 1 additional effective method (*e.g. latex, condom, diaphragm, or cervical cap*) at 4 weeks before starting thalidomide therapy, during therapy, and for 4 weeks after discontinuing thalidomide therapy even when there has been a history of infertility, unless due to hysterectomy or because the patients has been postmenopausal or has had no menses for at least 24 consecutive months.

3.7.8.3 Male patients must be counseled about the possibility that thalidomide may be present in semen. Men must use a latex condom every time they have sexual intercourse with a woman during therapy and for 4 weeks after discontinuing thalidomide, even if they have had a successful vasectomy.
3.7.8.4 The bottle label will bear:"Warning: This product is contraindicated in men and women of childbearing age. Thalidomide is an investigational new drug that can only be prescribed by a physician."

3.7.8.5 If secondary re-packaging is necessary then all bottles should bear a warning similar to the following: "Thalidomide must not be used by males and females who are sexually active."

3.7.8.6 In addition, all bottles should have affixed a warning label similar to the following: "This medication may cause drowsiness, alcohol may intensify this effect. Use caution when driving or operating machinery."

3.8 Pamidronate

3.8.1 Mechanism of Action: Pamidronate is an inhibitor of bone resorption and, in addition may interfere with the release, deposition of metastatic tumor deposits.

3.8.2 Toxicity: Fatigue, fever, diaarhea, nausea, arthralgias, and rarely hypocalcemia, phlebitis at the site of injection have been observed. In patients with multiple myeloma, the risk of renal dysfunction may be increased when a bisphosphonate is used in combination with thalidomide

02/03/04

3.8.3 Pharmaceutical Data: Pamidronate is supplied in 30, 60, and 90 mg vials as a freeze-dried powder. Reconstitution can be done in 10 mL of sterile water, then the appropriate volume of pamidronate will be mixed with normal (0.9 %) saline to a total volume of 250 mL. Pamidronate will be infused over 2 hours. If the reconstituted solution is not used immediately, it can be stored at temperatures between 36-46^{0}F and can be used for up to 24 hours.

Pamidronate is commercially available.

4.0 STAGING CRITERIA

4.1 Criteria for Diagnosis of Multiple Myeloma[42]

4.1.1 Major Criteria

Plasmacytomas on tissue biopsy.

Bone marrow plasmacytosis with > 30% plasma cells.

Monoclonal globulin spike on serum electrophoresis exceeding 3.5 gm% for G peaks or 2.0 gms for A peaks. $\geq$ 1.0 gm/24 hours of kappa or lambda light chain excretion on urine electrophoresis in the absence of amyloidosis.

4.1.2 Minor Criteria

Bone marrow plasmacytosis 10% - 30%.

4.1.3 Monoclonal globulin spike present, but less than the levels defined above.

4.1.4 Lytic bone lesions.

4.1.5 Normal lgM less than 50 mg%, lgA less than 100%, or lgG less than 600 mg%.

4.1.6 Diagnosis will be confirmed when any of the following features are documented in symptomatic patients with clearly progressive disease. The diagnosis of myeloma requires a minimum of 1 MAJOR + 1 MINOR CRITERION or 3 MINOR CRITERIA which must include a+b, i.e.:

4.1.6.1 l+b, l+c, l+d (l+a not sufficient)

4.1.6.2 ll+b, ll+c, ll+d

4.1.6.3 lll+a, lll+c, lll+d

4.1.6.4 a+b+c, a+b+d

4.1.7 The presence of certain nonspecific disease features will support the diagnosis, particularly if of recent onset.

4.1.7.1 Anemia

4.1.7.2 Hypercalcemia

4.1.7.3 Azotemia

4.1.7.4 Demineralization and compression fractures

4.1.7.5 Hypoalbuminemia

4.1.8 Great care must be taken to distinguish between active myeloma as defined above and MGUS or indolent/smoldering myeloma (see Section 4.2).

4.2 Criteria for Monoclonal Gammopathy of Undetermined Significance (MGUS), Indolent Myeloma and Smoldering Myeloma (Stage I or IIA)[40]

4.2.1 MGUS

4.2.1.1 Monoclonal gammopathy
4.2.1.2 M-Component level

4.2.1.2.1 IgG $\leq$ 3.5 gm%

4.2.1.2.2 IgA $\leq$ 2.0 gm%

4.2.1.2.3 BJ $\leq$ 1.0 gm/24 hours

4.2.1.3 Bone marrow plasma cells < 10%

4.2.1.4 No bone lesions

4.2.1.5 No symptoms

4.2.2 Indolent Myeloma Criteria as for myeloma (see Section 4.2) except (all of the following):

4.2.2.1 No or only limited bone lesions ($\leq$ 3 lytic lesions); no compression fractures.

4.2.2.2 M-component levels

4.2.2.2.1 IgG < 7 gm%

4.2.2.2.2 IgA < 5 gm%

4.2.2.3 No symptoms or associated disease features, i.e.:

4.2.2.4 Performance status > 50%

4.2.2.4.1 Hemoglobin > 10 gm%

4.2.2.4.2 Serum calcium normal

4.2.2.4.3 Serum creatinine < 2.0 mg%

4.2.2.4.4 No infections

4.2.3 Smoldering Myeloma Criteria as for indolent myeloma except:

4.2.3.1 NO bone lesions

4.2.3.2 Bone marrow plasma cells ≤ 30%

4.3 Assessment of Tumor Mass

4.3.1 High Tumor Mass (Stage III) One of the following abnormalities must be present:
4.3.1.1 Hemoglobin < 8.5 gm%, hematocrit < 25 vol.%, or

4.3.1.2 Serum calcium > 12 mg%, or

4.3.1.3 Very high serum or urine myeloma protein production rates:

4.3.1.3.1 IgG peak > 7 gm%

4.3.1.3.2 IgA peak > 5 gm%

4.3.1.3.3 Bence Jones protein > 12 gm/day (24 hours), or

4.3.1.3.4 > 3 lytic bone lesions on bone survey (bone scan not acceptable)

4.3.2 Low Tumor Mass (Stage I) ALL of the following must be present:

4.3.2.1 Hemoglobin > 10.5 gm% or hematocrit > 32 vol.%

4.3.2.2 Serum calcium normal

4.3.2.3 Low serum myeloma protein production rates:

4.3.2.3.1 IgG peak < 5 mg%

4.3.2.3.2 IgA peak < 3 gm%

4.3.2.3.3 Bence Jones protein < 4 gm/day (24 hours)

4.3.2.3.4 Bone lesions scaled 0 (none) or 1 (osteoporosis)

4.3.3 Intermediate Tumor Mass (Stage II)

All other patients who do not qualify specifically for high or low tumor mass categories are considered to have intermediate tumor mass.

4.4 Assessment of Renal Status

4.4.1 A = Good Renal Function (creatinine $\leq$ 2.0 mg%)

4.4.2 B = Poor Renal Function (creatinine > 2.0 mg%)

4.5 Assessment of Myeloma Cell Mass

Cell Mass Category		STAGE III High	STAGE I Low	STAGE II Intermediate
# of myeloma cells		> 1.2 x $10^{12}/m^2$	< 0.6 x $10^{12}/m^2$	0.6-1.2 x $10^2/m^2$
Requirements		One of: A,B,C,D	All of: A,B,C,D	Neither High or Low
Hemoglobin (gm%) (pretransfusion)	A	< 8.5	> 10.5	$\geq$ 8.5
Serum calcium (mg%)	B	> 12	Normal	$\leq$ 12
M-Component	C	IgG > 7 gm% IgA > 5 gm% BJ > 12 gm/day	< 5 < 3 < 4	$\leq$ 7 $\leq$ 5 $\leq$ 12
Bone lesions (on survey only; bone scan not acceptable)	D skeletal	Scaled 3 (> 3 lytic lesions)	scaled 0 or 1 (no lesions or osteoporosis only)	Scaled 0, 1, or 2

NOTE: The staging of patients with IgD or IgE monoclonal spikes is based upon other (non M-component) criteria.

5.0 PATIENT ELIGIBILITY

5.1 Patients with multiple myeloma will be eligible.

5.2 Patients with smoldering myeloma are eligible if there is evidence of progressive disease requiring therapy ($\geq$ 25% increase in M protein levels or Bence Jones excretion; Hgb $\leq$ 10.5 g/dl; frequent infections; hypercalcemia; rise in serum creatinine above normal on two separate occasion)

5.3 Patients with non-quantifiable monoclonal proteins are eligible provided they meet other

criteria for multiple myeloma, or smoldering myeloma.

5.4 Patients with the following reponse/status after induction therapy and after no more than 3 prior chemotherapy regimens (other then Decadron) are eligible:

5.5 Responding, or stable MM , and less or equal to 40% myelomatous involvement in the bone marrow biopsy specimen.

5.6 Patients with Waldenstrom's macroglobulinemia are not eligible.

5.7 Less than 18 months since diagnosis.

5.8 Patients must be $\leq$ 65 years old at the time of enrollment.

5.9 A KPS of > 70 % is required.

5.10 No contraindication to the collection of a minimum of 4 x 10^6 CD34+ cells/kg by apheresis.

5.11 All patients must have signed a voluntary, informed consent in accordance with institutional and federal guidelines.

5.12 Adequate hepatic function as demonstrated by bilirubin, $\leq$ 1.5 mg/dl, and SGOT and SGPT < 2.5 x upper limits of normal.

5.13 Adequate renal function as demonstrated by: creatinine of $\leq$ 1.4 mg/dl and measured creatinine clearance of > 60 cc/min.

5.14 Absolute neutrophil count of > 1000/µl, platelet count of > 100,000/µl.

5.15 Cardiac ejection fraction $\geq$ 50% by MUGA scan and/or by echocardiogram.

5.16 Adequate pulmonary function as demonstrated by FEV1 > 60% and DLCO > 50% of predicted lower limit.

5.17 Hepatitis B antigen, Hepatitis C RNA and HIV antibody tests negative.

5.18 No other medical, or psychosocial problems which in the opinion of the primary physician or principal investigator would place the patient at unacceptably high risk from this treatment regimen.

5.19 Females of reproductive age not using adequate birth control measures/ or who are pregnant are not eligible.

5.20 History of other malignancies within the last 5 years, except for non-melanoma skin cancer and in situ carcinoma of the cervix.

5.21 Patients should have finished their prior chemotherapy, or radiation treatment at least 4 weeks before cyclophosphamide priming.

5.22 Pre-treatment tests must have been performed within 6 weeks prior to initiation of cyclophosphamide.

6/14/99 5.23 Known hypersensitivity to Filgrastim or to E. coli derived proteins is an exclusion.

6.0 TREATMENT PLAN

6.1 Pre-treatment Evaluation

6.1.1 History and physical examination.

6.1.2 Radiographic evaluation: MRI of the entire spine and pelvis and skeletal survey will be performed.

6.1.3 Chest X-ray.

6/14/99 6.1.4 Pulmonary function tests

6.1.5 MUGA scan or echocardiogram.

6.1.6 CBC, differential count, platelet count, PT, PTT, SMA 18, Mg.

6.1.7 Urine analysis.

6/14/99 6.1.8 24 urine collection for total protein, protein electrophoresis, immune electrophoresis, and creatinine clearance.

6/14/99 6.1.9 Hepatitis panel, HIV antibody, HSV and CMV antibody.

6.1.10 Unilateral bone marrow biopsy and aspirate for morphology, cytogenetics/FISH. Aspirate for HUMARA (females only).

6/14/99 6.1.11 Peripheral blood for FISH (including clonal hematopoiesis markers), gene rearrangement, and for lymphocyte subset analysis.

6/14/99 6.1.12 Serum protein electrophoresis, quantitative serum immunoglobulins, serum immunofixation electrophoresis

6.1.13 Beta-2 Microglobulin level.

6.1.14 Urine pregnancy test.

6.1.15 HLA, ABO and Rh typing.

6.1.16 Double lumen Hickman catheter will be placed ($\geq$ 12 French catheter).

6.2 Patient Registration

After all pre-treatment evaluations have been performed patients can be entered on study. Eligibility requirements must be reviewed by a member of the department of Biostatistics and the principal investigator. Patients may be screened for eligibility by calling the Department of Biostatistics at extension 2468.

6.3 Harvesting and Cryopreservation of Stem Cells

6/14/99 Cyclophosphamide 1.5 gm/m^2 (based on actual body weight) will be administered with 500 cc D5/W over 2 hours. Filgrastim 5 μg/kg (based on actual body weight) bid, sq or iv will be administered starting 24 hours after cyclophosphamide and continue through the completion of apheresis (total daily Filgrastim dose equals 10 μg/kg/day).

Beginning day 10, provided that the peripheral blood white cell count is > 1000/μL and rising**,** apheresis will commence and continue until a minimum of 4 x 10^6 CD34+ cells/kg are collected. For patients with good CD34+ cells yields during apheresis (once the minimum CD34+ requirements are secured) a target of $\geq$ 10 x 10^6 will be set and no more than 10 aphereses are recommended.

Filgrastim will continue to be given daily; Filgrastim dose will be held if the total white cell count is $\geq$ 80,000/μl. If the yield from the first 3 days of apheresis is < 1 x 10^6/kg, the dose of Filgrastim will be increased to 10 μg/kg, bid.

The collections will last 4 hours, or until a volume of 12 liters has been processed. Peripheral stem cells will be processed and cryopreserved following standard methods at the City of Hope National Medical Center.

6.4 <u>High-Dose Chemotherapy</u>

6.4.1 <u>Cycle 1: Melphalan</u>

6.4.1.1 DAY -1

Admission, history and physical, SMA 18, Mg, CBC, differential and PLT count, urine analysis. Review of required laboratory, screening and radiographic data, signing of consent form.

Prophylactic IV, or p.o. fluconazole at 200 mg daily will be given to patients with AGC < 1000/μl.

10/10/02 Intravenous hydration with normal saline at 200 cc/hr and KCL 15 MeQ/1 will be started. After 6 hours of hydration melphalan 150 mg/m^2 will be infused in ≤ 30min. IV hydration will continue with normal saline at a rate of 200 cc/hr and KCL 15 MeQ/l for a total of at least 24 hours.

Appropriate intravenous antiemetics will be given.

When calculating chemotherapeutic dose, ideal body weight will be used (See appendix C).

6.4.1.2 DAY 0

Half of the previously collected CD34+ cells will be reinfused.

6.4.1.3 Day +1

Filgrastim 5 µg/kg daily, IV, or SQ will be started. Continue with Filgrastim until AGC > 1000/µl for 3 consecutive days

Patients will be supported through iv hydration and TPN, red cell and platelet transfusions as needed. CBC, PLT and SMA7, SMA 12, and Mg and weekly chest x-ray as well as the necessary fever workup will be done. Patients can be followed in the outpatient unit unless they develop neutropenic fever, uncontrollable diarrhea or other problems requiring inpatient care. Inpatient care will be provided in rooms equipped with HEPA filter and they will be in protective isolation. All blood products will be filtered and
08/99 radiated.

6.4.2 Cycle 2:

10/10/02 6.4.2.1 Busulfan and Cyclophosphamide will start a minimum of 12 weeks and a maximum 18 weeks from cycle number 1.

6.4.2.2 DAY -8

Admission, history and physical, SMA18, Mg, CBC, differential and PLT count, urine analysis. Review of required laboratory, screening and radiographic data, check for signing of consent form. On day -8 dilantin loading dose at 1000 mg iv will be given.

Prophylactic IV, or p.o. fluconazole at 200 mg daily will be given to patients with AGC < 1000/µl.

6.4.2.3 DAY -7 through -3

Continue with dilantin 300 mg iv, daily x five days.

6/14/99 6.4.2.4 DAY -7 through –4

10/10/02 IV busulfan at 0.8 mg/kg Q 6 hr will be given for a total of 16 doses with each dose infused over 2 hours. A total of 7 blood draws, approximately 2 tablespoons of blood in total will be drawn with the first dose of busulfan. Five (5) milliliters of peripheral blood will be collected in green-top (sodium heparin) tubes at the following times; pre-dose, just prior to the end of the infusion, then 15 minutes, 30 minutes, 1, 2, and 4 hours after the end of the dose. The last sample will be

obtained just prior to the start of dose 2.

When calculating chemotherapeutic dose (including mesna) , ideal body weight will be used (See appendix C).

6.4.2.5 DAY -3

Cyclophosphamide at 60 mg/kg iv in 500 cc D5W will be given over 2 hours. MESNA 25 mg/kg as bolus is given prior to cyclophosphamide and then MESNA 120 mg/kg over 48 hours as continuous infusion is administered.

When calculating chemotherapeutic dose, ideal body weight will be used.

02/22/01 6.4.2.6 DAY -2

Processing of samples consists of adding 1 ml of whole blood to specially prepared tubes containing a trapping agent, which prevents the degradation of 4-hydroxycyclophosphamide (4HC) ex vivo. Due to the rapid breakdown of 4HC ex vivo, samples must be processed within 2 minutes. Therefore, a protocol nurse must be present prior to drawing blood. The remainder of the blood not used for 4HC measurement will be collected in green-top (sodium heparin) tubes and centrifuged at 1500xg to separate plasma from whole blood. Plasma will be stored frozen at _ -20_C until later batch analysis for cyclophosphamide.

6.4.2.7 DAY 0

Half of the previously collected CD34+ cells will be reinfused. and Filgrastim 5 µg/kg daily, IV, or SQ will be started. Continue with hydration as needed. Continue with Filgrastim until AGC > 1000/µl for 3 consecutive days.

Patients will be supported through iv hydration and TPN, red cell and platelet transfusions as needed. Daily CBC, PLT and SMA7, every Monday, Wednesday, Friday SMA 18, and Mg and weekly chest x-ray, biweekly PT, PTT as well as the necessary fever workup will be done. Patients can be followed in the outpatient unit unless they develop neutropenic fever, uncontrollable diarrhea or other problems requiring inpatient care. The inpatient care will be provided in rooms equipped with HEPA filter and they will be in protective isolation. All blood products will be filtered and radiated according to standards at the COH.

6.4.4 Maintenance Therapy

02/22/01 6.4.4.1 Phase I: Patients with responding, or stable disease following sequential high-dose chemotherapy will receive maintenance therapy with alpha interferon. Treatment with interferon will begin at least 6 weeks and no later then 20 weeks from day 0 of the second cycle of high-dose chemotherapy provided that an AGC of > 1000/µl and a platelet count of > 100,000/µl has been reached.

Interferon will be given at 3.0 million units/m^2 on Mondays, Wednesdays and Fridays, SQ. Interferon will be administered for 3 years.

6/14/99 Aredia 90 mg iv every 4 weeks and will be continued till progression.

01/07/00 6.4.4.2 Phase II: Patients not in complete remission by 6 months from day 0 of the second high-dose therapy cycle will be started on Thalidomide 200 mg for 1 week, 300 mg for the next week, and then will be 04-01/07/00 receiving a target dose of 400 mg. Patients will be receiving Thalidomide for a maximum of 1 year, or for 3 months after a documented concersion to complete remission (whichever is sooner), unless they progress, or cannot tolerate Thalidomide in spite of dose adjustments required due to toxicities.

6.5 Dose Adjustments

6.5.1 Melphalan

There will be no dose adjustments for melphalan

6.5.2 Busulfan and cyclophosphamide

10/10/02 Busulfan and cyclophosphamide will be given at the earliest 12 weeks and at the latest 18 weeks from day 1 of the melphalan cycle. Patients requiring further delaying of treatment will be taken off study.

There will be no dose adjustments for Busulfan and Cyclophosphamide.

Pre-cycle 2 requirements should be met prior to initiating the second cycle.

01/07/00 6.5.3 Thalidomide will be started at 200 mg given orally, daily for one week and the dose will be escalated to 300 mg for the following week, and to 400 mg from the third week on. For grade >2 neurotoxicity, or > grade 2 hematologic toxicity, or other > grade 2 toxicities the dose of Thalidomide will be held for one week and then restarted at a dose which is 100 mg/dose lower than the dose causing > grade 2 toxicities. Re-escalation to a higher dose (maximum 400 mg/day) is allowed. Any patients who cannot tolerate the lowest 200 mg/day dose after being off therapy for 4 weeks due to persistent > grade 2 toxicities will not receive any further dose of Thalidomide.

6.5.4 Interferon

Patients experiencing weight loss, fatigue, flue like symptoms or any other > grade 2 toxicity will have their interferon dose reduced by 50%. If after 2 weeks symptoms persist a 2 week break will commence. Then, interferon will be restarted at the 50% dose for two weeks and subsequently will be re-escalated to full dose. A second adjustment to 50% dose is permissible (to 25 % of the starting dose) and patients could continue on the reduced dose of interferon for the duration of study. However, should a patient require a third reduction of interferon he/she should be taken off therapy.
01/07/00

6.5.5 General Side Effects

The side effects associated with the use of individual drugs: cyclophosphamide, MESNA, Filgrastim, melphalan, busulfan, alpha interferon, and Thalidomide are listed under section 3.

6.5.6 Pamidronate

There will be no dose adjustments for pamidronate.

7.0 EVALUATION AND TOXICITIES

Physical evaluation, laboratory and radiographic evaluation will be performed as outlined in the treatment regimen. When evaluating toxicity a modified NCI autologous bone marrow toxicity scale will be used.

06/14/99
09/27/99
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02/22/01

8.0 STUDY PARAMETERS

02/03/04

	Within 6 Wks Pre-Cytoxan	Pre-Cycle 1	Cycle 1	Pre-Cycle 2	Cycle 2	Q30 days**	Q 6 mos[#]	Q yr[+]
History and Physical	X	X		X	X	X		X
CBC, DIFF, PLT/SMA7	X	X	Daily	X	Daily	X		X
SMA 18, MG[++]	X	X	M,W,F	X	M,W,F	X		X
PT, PTT	X	X		X	QO Week			X
02/03/04 Urinalysis Creat Clearance	X			X		X[&]		
Chest X-ray	X		Q Week	X	Q Week			
MRI of bones, bone x-rays	X						X **	X
SPEP, QSIEP, Beta-2 Microglobulin	X			X				X
24 hr urine for PEP, IEP, total protein, creatinine clearance	X			X				X
MUGA Scan/ or echo	X			X				X **
EKG	X			X				X **
PFT	X							
AB, RH, HLA Typing	X							
BM, ASP, BX, for morphology, cytogenetics FISH, gene rearrangements/clonal hematopoiesis, vascular density	X					X[‡]	X[#]	X
Blood for Cytogenetics/FISH and clonal hematopoiesis	X	X[++]					X[#]	X
PBPC product for cytogenetics, FISH clonal hematopoiesis	X	X[++]						
Urine Pregnancy Test (For female patients)	X							
HIV, Hepatitis Panel to include A,B,C; Herpes and CMV Titer	X							
T cell subsets by flow cytometry NK cell activity	X					X *		X **

[#]Counting from day 0 of cycle 2;
*Pre-interferon, at 6 months from day 0 of cycle 2 (pre-Thalidomoide) and 12 months from day 0 of cycle 1;
**During the first year;
[+] For the first 3 years;
[++] First day of apheresis
[‡] At 30 days from day 0 of cycle 2
02/03/04 [&] If receiving thalidomide

9.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

The following definitions will be used for this study. Response can be defined as a 50% or better reduction in serum myeloma protein production and further categorized as completed and partial. (Adopted from the ongoing Intergroup Study)

9.1 Response Gradations:

9.1.1 Complete response: Defined as the absence of bone marrow or blood findings of multiple myeloma on at least 2 measurements at a minimum of a 6 week interval. Thus all evidence of serum and urinary M-components must disappear on electrophoresis as well as by immunofixation studies. The follow-up bone marrow may not contain more than 3% plasma cells on aspiration or core biopsy and no evidence of increasing anemia. Skeletal X-rays must either show recalcification or no change in osteolytic lesions.

9.1.2 Partial Response: Sustained decrease of the monoclonal serum protein by $\geq$ 50% reduction) on at least two measurements of at least 6 week interval. A response is clear-cut for patients who achieve a 75% or greater reduction in the serum myeloma protein concentration without evidence of increasing anemia. Responding patients must also have a sustained decrease in 24-hour urine M-component to less of the initial prestudy value, and to less than 0.2 gm/day on at least two measurements at three-week intervals. For a response to be confirmed, a marked reduction in both serum myeloma protein and in Bence-Jones protein excretion must be present. Bone marrow plasma cells should be reduced by at least 50% from the pretreatment level.

9.1.3 In all complete, and responding patients, the size and number of lytic skeletal lesions must not increase, and the serum calcium must remain normal. Any of the following ancillary data will support the conclusion that an objective response has occurred, but are not required to confirm response: 1) recalcification of lytic skull or pelvis lesions (occurs in about 20% of responding patients with lytic lesions); 2) significant increase in depressed normal immunoglobulins, as in lgM increments exceeding 20 mg%, lgA exceeding 40 mg%, and lgG exceeding 400 mg% (occurs in about 15% of responding patients); 3) fall in the level of the serum beta-2 microglobulin to the normal range (less than 1.0 mg/dl). However, it is well established that IFN can increase the B_2M levels, which does not signify relapse.

9.2 Stable: Patients with 25 - 49% tumor mass regression without new symptoms or signs of myeloma with declines in the serum myeloma protein production of less than 25% of the pretreatment level will be considered to have no response.

9.3 Plateau State: Is defined as a lack of progression (change of $\leq$ 25% myeloma protein) for a minimum duration of 3 months.

9.4 Progression: Patients with a > 25% increase in myeloma protein production or other signs of disease such as hypercalcemia, etc.

9.5 Relapse: This is defined by the unequivocal objective evidence and constitutes the earliest of any of the following: 1) an increase by more than 100% from the lowest level of the serum myeloma protein production; 2) an increase above the response level of the myeloma peak (i.e., relapse to more than 25% of the baseline myeloma protein production); 3) reappearance of

the myeloma peaks that had disappeared with the treatment; 4) definite increase in the size and number of lytic bone lesions recognized on radiographs. Compression fractures per se do not constitute a relapse.

9.6 Performance Status: Karnofsky Performance status will be recorded.

10.0 REPORTING DATA

All primary data will be maintained by the Department of Biostatistics, City of Hope National Medical Center. This includes on study flow sheets, consent forms and off-study forms.

01/07/00 **11.0 STATISTICAL CONSIDERATIONS**

11.1 Sample Size

The primary goal of this phase II study is to assess the feasibility and toxicities of administering two cycles of sequential high-dose chemotherapy consisting of melphalan and subsequent busulfan and cyclophosphamide followed by autologous stem cell support, alpha interferon, and thalidomide in patients with multiple myeloma. Feasibility will be defined as the ability to receive all IL-2
01/07/00 treatments. The secondary objectives are to analyze response rate, progression-free and overall survival following receipt of this treatment regimen; and to assess the feasibility of adding Thalidomide for patients not yet in complete response (CR) at 6 months and the subsequent CR rate received.

03/13/02 An accrual goal of 70 patients will be set, allowing us to estimate the rate of feasiblely administering this treatment regimen with a maximum standard error of
01/07/00 7%. Based on our past experience, it is estimated that approximately 18 patients (35%) will have achieved a CR by 6 months, so that approximately 32 patients will go on to receive Thalidomide.

Based on the current referral patterns to the Department of Hematology and Bone Marrow Transplantation, it is expected that approximately 20 patients per year
03/13/02 will be eligible for this study. With a goal of 70 patients, approximately 3 ½ years of accrual will be required. As the majority of relapses are expected to occur within 3 years or less, the study will continue for 3-4 years to allow sufficient follow-up for the secondary goal of analyzing disease progression and survival as well.

11.2 Toxicity Grading

Hematologic suppression has traditionally been accepted as a primary endpoint for analysis of toxicity in investigational agents and novel drug combinations in oncology. Nevertheless, autologous peripheral stem cell transplantation has ameliorated this toxicity significantly, allowing for high-dose chemotherapy. We will utilize the Division of Cancer Treatment (DCT)/National Cancer Institute (NCI) Common Toxicity Criteria for evaluating toxicity of all organ systems, with the exception of the hematologic and gastrointestinal system, where we will follow the modified guidelines for studies involving bone marrow transplantation as recommended by the Cancer Therapy Evaluation Program (CTEP) (Appendix D).

11.3 Early Stopping Rules

10/10/02 We have monitored the rate of treatment related death and so far, such death rate is 1.8% after treating 54 patients. The study statistics call for a total of 70 patients for accrual. Hence, if there is more then one additional VOD related treatment associated mortality in the next 10 patients, or if there are more then two cases within the next 20 patients who's demise is related to VOD associated treatment related side effects, this study will be stopped.

At the same time we will monitor the study for early stopping due to inability to receive the proposed treatment scheme. Historically the overall feasibility rate with IL-2 treatment has been estimated to be 65%. Inability to receive the full treatment regimen may be due to any of the following causes: grade 3 or greater toxicities, intolerance, or disease progression within one year. If at any point in the monitoring process there is a 95% or greater probability that the true feasibility rate is less than 65%, then the trial will be stopped early. This would occur if the following numbers of inability to tolerate treatment regimen were observed:

11.4 Criteria for Success

03/13/02 If the study accrues to the full 70 patients, the trial will be considered a success on the primary endpoint if an overall feasibility rate of 65% or greater is observed. If the estimated feasibility rate in the study group is equal to 65% then the 95% confidence interval would range from 52% to 78%, so that a minimum feasibility rate of 52% is ensured with 95% confidence.

01/07/00 Historically we have observed that approximately 10% of those patients who are not in CR at 6 months reach a CR after 6 months without administration of Thalidomide. If 10 out of the 32 patients (31%) receiving Thalidomide on the current study can be converted to CR after 6 months, then this approach will be considered a success, with the 95% confidence interval for late CR conversion ranging from 15% to 47%.

03/13/02 After treating the first 50 patients, the actual number requiring thalidomide is only 46%. In order to achieve the initial targeted number of 32 patients, not in CR, and requiring thalidomide (thereby being able to assess the feasibility of tolerating thalidomide together with alpha-interferon as well as the ability of thalidomide to induce complete response conversion). We request that the targeted accrual be increased to 20, hence, an additional 9 patients, not in CR, can be treated with thalidomide for a total of 32 patients.

11.5 Analytic Plan

The primary endpoint of feasibility will be estimated as a proportion along with the 95% confidence interval for the binomial. If our accrual goal is met with no premature termination due to significant toxicity, a secondary goal of assessing response, progression-free survival (PFS), and overall survival (OS) will be carried out. Survival estimates will be made using the product-limit method of Kaplan and Meier, with 95% confidence limits calculated using the logit transformation. These estimates will be contrasted with the historical rates of 81% PFS and 85% OS at two years, although formal hypothesis testing will not

01/07/00 be conducted. It is hoped that PFS and OS will be further improved with the addition of Thalidomide.

While the primary estimates will be for the entire study group, survival estimates will also be carried out for subgroups stratified by bone marrow involvement (<=10% versus >10%), by remission status (CR/PR versus Stable Disease), and by cytogenetic abnormality (yes versus no), as these are known to be important prognostic factors. Additional descriptive analyses assessing the incidence of hepatic and other toxicities was a correlate of busulfan and(if feasible) cyclophosphamide pharmacokinetics will also be carried out.

01/07/00 **12.0 GENDER AND ETHNICITY STATEMENT**

The City of Hope has a plan in place to increase minority recruitment to our studies in compliance with the National Institute of Health policy for recruitment of women and minorities.

The table below shows the distribution by sex and race of the patients accrued to therapeutic clinical studies at City of Hope for the past five years with the same primary site of disease targeted for this protocol (breast). Our goal is to maintain our high accrual of women while continuing to increase the accrual of minority subjects.

01/07/00

Accrual Goal by Sex and Ethnicity								
		By Sex		By Ethnicity				
Site	Accrual Goal	Female	Male	White	Hispanic	Black	Asian/ Other	Unknown
Multiple Myeloma	367	172 (47%)	195 (53%)	213 (58%)	66 (18%)	70 (19%)	14 (4%)	4 (1%)

13.0 ETHICAL AND REGULATORY CONSIDERATIONS

This study is to be approved by the Institutional Review Board of the City of Hope. All patients will have signed an informed consent for participation in research activities, and will have been given a copy of the Experimental Subject's Bill of Rights.

When results of this study are reported in medical journals or at meetings, identification of those taking part will be withheld. Medical records of patients will be maintained in strictest confidence, according to current legal requirements. However, they will be made available for review, as required by the Food and Drug Administration (FDA) or to other authorized users such as the National Cancer Institute (NCI) under the guidelines established by the Federal Privacy Act.

01/07/00 **14.0 BIBLIOGRAPHY**

1. Riedel DA. In: Epidemiology and biology of multiple myeloma. GI Obrams and M Potter (eds.), Springer-Verlag, New York, 1991, pp. 13-16

2. Kyle RA. Epidemiology and biology of multiple myeloma. GI Obrams and M Potter (eds.), Springer-Verlag, New York, 1991, pp. 75-81

3. Barlogie B, Smallwood L, Smith T, Alexanian R. High serum levels of lactic

dehydrogenase identify a high-grade lymphoma-like myeloma. Annals Int Med 110:521-25, 1989

4. Barlogie B, Epstein J, Selvanayagam P, Alexanian R. Plasma cell myeloma -New biological insights and advances in therapy. Blood 73:865-79, 1989

5. Boccadoro M, Marmont F, Tribalto M, et al. Multiple myeloma: Alternating combination chemotherapy (VCMP/VBAP) is not superior to melphalan and prednisone (MP) even in high risk patients. J Clin Oncol 9:444-448, 1991

6. Barlogie B, Smith L, Alexanian R. Effective treatment of advanced multiple myeloma refractor to alkylating agents. N Engl J Med 310:1353-1356, 1984.

7. Sonneveld P, Durie BGM, Lokhorst HM, et al. Modulation of multidrug-resistant multiple myeloma by cyclosporin. Lancet 340:255-59, 1992.

8. Barlogie B. Hematopoietic cell transplantation for multiple myeloma. in Thomas ED, Blume GG and Forman SJ. eds, Hematopoietic Cell Transplantation, Boston, Blackwell Scientific Publishers, 1998:1003-1013

9. Cunningham D, Paz-Ares L, Gore ME, et al. High-dose melphalan for multiple myeloma: Long-term follow-up data. J Clin Oncol 12:764-768, 1994

10. Cunningham D, Paz-Ares L, Milan S, et al. High-dose melphalan and autologous bone marrow transplantation as consolidation in previously untreated myeloma. J Clin Oncol 12:759-763, 1994

11. Jagannath S, Barlogie B, Dicke K, et al. Autologous bone marrow transplantation in multiple myeloma: Identification of prognostic factors. Blood 76:1860-1866, 1990

12. Gore ME, Selby PJ, Viner C. Intensive treatment of multiple myeloma and criteria for complete remission. Lancet 2:879-855, 1990

13. Barlogie B, Alexanian R, Dicke K, et al. High-dose chemoradiotherapy and autologous bone marrow transplantation for resistant multiple myeloma. Blood 70:869-72, 1987

14. Bensinger WE, Buckner CD, Clift RA, et al. A phase I study of busulfan and cyclophosphamide in preparation for allogeneic marrow transplant for patients with multiple myeloma. J Clin Oncol 10:1492, 1992.

15. Barlogie B, Gahrton G. Bone marrow transplantation in multiple myeloma. Bone Marrow Transplant 7:71-79, 1991.

16. Bjorkstrand B, Ljungman P, Svensson H, et al. Allogeneic bone marrow transplantation versus autologous stem cell transplantation in multiple myeloma: a retrospective case-matched study from the Europian Group for Blood and Marrow Transplantation. Blood 88 : 4711-18, 1996

17. Attal M, Harousseau J, Stoppa A, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med 355:1844, 1996

18. Fermand J, Ravaud P, Chevret S, et al. High-dose therapy and autologous peripheral blood stem cell transplantation in multiple myeloma: Up-front or rescue treatment? Results of a multicenter sequential randomized trial. Blood 92:3131-36, 1998

19. Barlogie B, Jagannath S, Desikan KR et al. Total therapy with tandem transplants for newly diagnosed multiple myeloma. Blood 93:55-65, 1999

20. Bjorkstrand B, Svennson H, Goldschmidt H, et. al. Autotransplants in multiple myeloma-A registry study from the European Group for Bone Marrow Transplantation. Blood 94: S3154a, 1999

21. Attal M, Harosseau L, Facon T, et. al. Single versus double transplant in myeloma: A randomized trial of the "Intergroup Francais Du Myelome." Blood 94: S3152a, 1999

22. Tosi P, Cavo M, Zamagni E, et.al. A multicentric randomized clinical trial comparing single versus double autologous peripheral blood progenitor stem cell transplantation for patients with newly diagnosed multiple myeloma: Results of an interim analysis. Blood 94: s3155a, 1999

23. Reynolds C, Ratanatharaton V, Adams P, et. al. Allogeneic transplantation reduces disease progression compared to autologous transplantation for patients with multiple myeloma. Blood 94: S1548a, 1999

24. Molina A, McSweeney P, Maloney D, et. al. Non-myeloablative peripheral blood stem cell allografts following cytoreductive autotransplants for the treatment of multiple myeloma. Blood 94: S1551a, 1999

25. Giralt S, Weber D, Aleman A, et. al. Nonmyeloablative conditioning with fludarabine/melphalan for patients with multiple myeloma. Blood 94: 1549a, 1999

26. Vescio R, Han E, Schiller G, et al, Quantitative comparison of multiple myeloma tumor contamination in bone marrow harvest and leukapheresis autografts. Bone Marrow Transplant 18:103-110, 1996

27. Stewart A, Schiller G, Vescio R, et al. CD34 selection does not prolong disease free or overall survival in myeloma patients undergoing autologous stem cell transplant: results of a phase III study. Blood 94: S3151a, 1999

28. Attal M, Huget F, Schlaifer D, et al. Maintenance treatment with recombinant alpha interferon after autologous bone marrow transplantation for aggressive myeloma in first remission after conventional induction chemotherapy.

Bone Marrow Transplant 8:125-128, 1991.

29. Berenson J, Lichstenstein A, Porter L, et al. Long-term pamidronate treatment of advanced multiple myeloma patients reduces skeletal events. Myeloma Aredia Study Group. J Clin Oncol 16: 593-602, 1998

30. Dhodapkar M, Singh J, Mehta J, et al. Anti-myeloma activity of pamidronate in vivo. Br J Haematology 103:530-532, 1998

31. Vacca A, Di Loreto M, Ribatti D, et.al. Angiogenesis and plasma cell adhesion *molecu*les LFA-1, VLA-4, LAM-1, and CD44. Am J Hematol 50: 9-14, 1995

32. Vacca A, Ribatti D, Presta M, et.al. Bone marrow neovascularization, plasma cell angiogenesis potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. Blood 93: 3064-3073, 1999

33. Rajkumar s, Fonesca R, Witzig T, et.al. Bone marrow angiogenesis in patients achieving complete response after stem cell transplantation for multiple myeloma. Leukemia 13: 469-472, 1999

34. Berenson J, Lichstenstein A, Porter L, et al. Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. Myeloma Aredia Study Group. N. Engl J Med 334: 488-493, 1996

35. Singhal S, Mehta J, Eddlemon P, et.al. Marked anti-tumor effect from anti-angiogenesis with thalidomide in high-risk refractory multiple myeloma. Blood 92: S318a, 1998

36. Anderson KC, et al . Recent advances in the biology and treatment of multiple myeloma. ASH Education Program Book, ppp 63-88, 1998

37. Chesi M, Nardini E, Lim R, et al. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a nobel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. Blood 92: 3025-34, 1998

38. Knight SJL, et al. Development and clinical application of innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. Eur. J Hum Genet 4:1-8, 1997

39. Clark R , et al. Use of chromoprobe multiprobe system for the detection of hyperdiploidy in acute lymphoblastic leukemia., UK Cancer Cytogenetics Group Meeting, 3oth June, 1997

40. Allen RC, Zoghbi HY, Mosely et al, Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229, 1992

41. Mach-Pascual S, Legare RD, Lu D, et al, Predictive value of clonality assays in patients with non-Hodgkins lymphoma undergoing autologous transplant: A single institution study. Blood 91:4496, 1998

42. Delabesse E, Arai S, Kamoun P, et al. Quantitative non-radioactive clonality analysis of human leukemic cells and progenitors using the human androgen receptor (AR) gene. Leukemia 9:1578, 1995

43. Busque L, Mio R, Mattioli J, et al. Nonrandom X-inactivation patterns in normal females. Lyonization ratios vary with age. Blood 88:59, 1996

44. Gale RE, Wheadon H, Boulos P, Linch CD. Tissue specificity of X-chromosome inactivation patterns. Blood 83:2899, 1994

45. Somlo G, et al. Survival and toxicity update following tandem cycle melphalan and busulfan/cyclophosphamide with autologous peripheral blood progenitor cell rescue in multiple myeloma; lack of correlation between the incidence of hepatic veno-occlusive disease and busulfan pharmacokinetics. Blood 94: S 2571a. 1999

46. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 36:842-854, 1975

47. Quernin MH, Poonkuzhali B, Montes C, Krishnamoorthy R, Dennison D, Srivastava A, Vilmer E, Chandy M, Jacqz-Aigrain E. Quantification of busulfan in plasma by gas chromatography-mass spectrometry following derivatization with tetrafluorothiophenol. J. Chromatogr. B. Biomed. Sci. Appl. 709:47-56, 1998

48. Burton LC, James CA. Rapid method for the determination of ifosfamide and cyclophosphamide in plasma by high-performance liquid chromatography with solid-phase extraction. J. Chromatogr. 431:450-4, 1988

49. Anderson LW, Ludeman SM, Colvin OM, Grochow LB, Strong JM. Quantitation of 4-hydroxycyclophosphamide/aldophosphamide in whole blood. J. Chromatogr. B. Biomed. Appl. 667:247-57, 1995

APPENDIX E.

IMMUNOLOGIC CHARACTERISTICS, PHARMACOKINETIC STUDIES FISH AND HUMARA STUDIES

Samples of 7.5 mL of blood in green top tubes will be submitted for flow cytometric analysis with special emphasis for CD3, CD8, CD4, CD16, CD56 and CD57 marking. Samples will be obtained pre-interferon, at 6 months from day 0 of cycle 2 (pre-thalidomide) , and 12 months from day 0 of cycle 1. Details of NK cell functional analysis are still being worked out, and will be incorporated once the logistics (technician becomes available) are in place.

2. A total of 7 blood draws, approximately 2 tablespoons of blood in total will be drawn during the administration of the first dose of busulfan. Five (5) milliliters of peripheral blood will be collected in green-top (sodium heparin) tubes at the following times; pre-dose, just prior to the end of the infusion, then 15 minutes, 30 minutes, 1, 2, and 4 hours after the end of the dose. The last sample will be obtained just prior to the start of dose 2.

2.1 Processing of samples for busulfan determination will consist of centrifugation at 500xg to separate plasma from whole blood. Plasma will be stored frozen at 39_ -20_C until later batch analysis.

2.2 Busulfan concentrations in plasma will be determined using gas chromatography-mass spectrometry following derivatization with tetrafluorothiophenol.[47]

3. Samples for cytogenetics/FISH/Humara assays will be obtained as follows:

6.1 Fluorescence in situ hybridization and HUMARA studies

6.2 Pretreatment samples: Applies to all patients registered to this study.

6.2.1 Forty ml of peripheral blood (five sodium heparin or green top tubes) must be collected from each patient.

6.2.2 Pretreatment bone marrow: 6-8 ml of a pretreatment bone marrow aspiratefrom each patient collected into standard cytogenetic tubes (tubes supplemented with tissue culture medium) is requested.

2/22/01

4. Apheresis sample: Two to four ml of apheresis specimen will be collected and placed in standard cytogenetic tubes with tissue culture medium. This sample may be obtained from any of the apheresis collections.

6/14/99 5. Post-treatment samples:

5.1 Blood samples (forty ml of peripheral blood [five sodium heparin or green top tubes]) will be collected pre-interferon and at 12 months from day 0 of cycle 1 (melphalan) therapy from each patient.

5.2 Post-treatment bone marrow: 6-8 ml of a pretreatment bone marrow aspirate from each patient

collected into standard cytogenetic tubes (tubes supplemented with tissue culture medium) is requested.

5.3 The samples should be marked for same day delivery (room temperature) to Cytogenetics Research, Northwest Building, Room 2265. Vicki Bedell (ext. 2025) or Dr. Slovak (ext. 2313, also same extension for additional distribution for Dr. Yen) may be contacted regarding questions concerning specimen collection and processing.

5.4 To reduce the cost of this study, the assays will be performed in batches. Storage of the DNA (HUMARA) and fixed cell pellets (FISH) will follow the standard procedures for the cytogenetics laboratory, that is, samples will be stored in a
-80ºC locked freezer until ready for use.