A Trial of Busulfan, Melphalan, Fludarabine and T-Cell Depleted Allogeneic Hematopoietic Stem Cell Transplantation Followed by Post Transplantation Donor Lymphocyte Infusions for Patients with Relapsed or High-Risk Multiple Myeloma

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a two arm phase II trial to assess the progression-free and overall survival as well as the safety and efficacy of allogeneic hematopoietic stem cell transplantation using a preparative regimen with busulfan, melphalan, fludarabine, and anti-thymocyte globulin (ATG), and a T cell depleted stem cell transplant from a histocompatible related or unrelated donor in patients with relapsed or high-risk multiple myeloma. The administration of calculated doses of donor lymphocyte infusions post transplantation is designed to decrease the rate of relapse or to induce complete remissions in patients with residual disease following the allogeneic transplant. Candidates for this trial will include patients with multiple myeloma with relapsed disease following autologous stem cell transplantation who achieved at least a partial response (PR) following prior chemotherapy (cohort 1) and patients with high-risk cytogenetics at diagnosis achieving at least a very good partial response (VGPR) following prior autologous stem cell transplantation (cohort 2). Patients with plasma cell leukemia or patients achieving less than PR/VGPR are not eligible for this trial. Hematopoietic stem cell donors for this trial will include individuals who are 10/10 HLA matched or one antigen or allele mismatched at the HLA-A, B, C, DRB1 or DQB1 locus, as defined by high resolution methods. Donors who are 8/10 HLA matched with an antigen or allele mismatched at HLA-DQB1 and at one other locus will also be eligible for the trial. All patients will be conditioned for transplantation with busulfan (Busulfex®) (0.8 mg/Kg/dose Q6H x 10 doses), melphalan (70 mg/m^2/day x 2 doses) and fludarabine (25mg/m^2/day x 5 doses). Doses of busulfan and melphalan will be adjusted according to ideal body weight, busulfan will be adjusted according to first dose pharmacokinetic studies and doses of fludarabine will be adjusted according to measured creatinine clearance. Patients will also receive ATG (Thymoglobulin®) prior to transplant to promote engraftment and to prevent graft-versus host disease post transplantation. The preferred source of stem cells will be peripheral blood stem cells (PBSC) mobilized by treatment of the donor with G-CSF for 5-6 days. PBSC will be isolated, and T-cells depleted by positive selection of CD34+ progenitor cells, using the CliniMACS Cell Selection System. The CD34$^+$ T-cell depleted peripheral blood progenitors will then be administered to the patients after they have completed cytoreduction. If the use of CD34$^+$ PBSC is not possible, the alternative graft will consist of bone marrow derived stem cells T-cell depleted by CD34 selection of bone marrow using the Milteni CliniMACS device. This system has recently been validated and implemented for T-cell depletion of bone marrow, and will replace the Lectin/SRBC T-cell depletion procedure. No drug prophylaxis against GvHD will be administered post transplant. All patients will also receive G-CSF post-transplant to foster engraftment. Patients will also receive donor lymphocyte infusions following transplantation derived from the same hematopoietic stem cell donor. Donor lymphocytes will be administered prophylactically at calculated doses of 5x10^5 CD3$^+$ cells/kg 4-6 months and a second dose 5x10^5 CD3$^+$ cells/kg 3-4 months post initial infusion to recipients from HLA-matched donors. The second infusion will be administered in the absence of graft-versus-host disease. A third infusion of 1x10^6 CD3$^+$ cells/kg can be administered 2-4 months following the second infusion, if the patient did not achieve a complete remission and lacks signs of graft-versus-host disease. Recipients of HLA-mismatched allografts will be infused with donor lymphocytes preemptively at disease relapse or progression of disease, if no complete remission was obtained following the transplant. These patients will then receive 1x10^5 CD3$^+$ cells/kg at diagnosis of relapse or progression, but no sooner than 4-6 months post transplantation. A second infusion of 5x10^5 CD3$^+$ cells/kg will be administered 1-3 months following the first infusion provided the patient lacks signs of graft-versus-host disease. A third

infusion of $1x10^6$ $CD3^+$ cells/kg can be administered 3-4 months following the second infusion, if the patient did not achieve a complete remission and lacks signs of graft-versus-host disease. Recipients of HLA-matched allografts will be treated preemptively at diagnosis of relapse or progression and according to the treatment plan described for those recipients of HLA-mismatched allografts. Patients will be monitored post transplant for donor engraftment, chimerism, incidence and severity of acute and chronic GvHD, regimen-related toxicity, characteristics of hematopoietic and immune reconstitution; transplant related and relapsed related mortality, overall and disease-free survival.

A maximum of 30 patients per cohort will be accrued to the study and the accrual period is expected to last 3 years with an additional 24 months of follow up after the accrual has been completed. The primary endpoint of the study is to assess one-year progression free survival from the time of transplant. An event is defined as a disease progression or death. For the relapsed multiple myeloma cohort, a single stage design that differentiates between one-year PFS rates of 0.30 and 0.54 will be used to assess treatment efficacy. For the high-risk multiple myeloma cohort, a single stage design that differentiates between one-year PFS rates of 0.45 and 0.69 will be used to assess treatment efficacy.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

To study in a prospective phase II trial the progression-free survival, efficacy and safety of a chemotherapy-based cytoreduction using busulfan, melphalan, fludarabine, and ATG with T-cell depleted hematopoietic stem cell transplants from histocompatible related and unrelated donors, in patients with relapsed or high-risk multiple myeloma. This trial also incorporates prophylactic or preemptive post transplantation administrations of calculated doses of donor lymphocyte infusions derived from the same hematopoietic stem cell donor.

Primary objectives of this trial are:

1. To determine the rates of progression-free (PFS) and of overall survival (OS) at 12 months post transplant.

Secondary objectives of this study are:

1. To assess the transplant-related morbidity and mortality for patients with multiple myeloma.
2. To assess the incidence of and severity of acute and chronic GvHD.
3. To compute the current multiple myeloma free survival curve in order to account for patients who relapse and are restored to remission through DLI.
4. To explore the associations between progression free survival and the CT antigens - CT7, CT10, MAGE-A3 and NY-ESO-1.

3.0 BACKGROUND AND RATIONALE

3.1. Advanced Multiple Myeloma

Multiple Myeloma (MM) is a malignant disease of plasma cells that accumulate in bone marrow, leading to bone destruction and bone marrow failure [1-2]. The disease accounts for approximately 10% of all hematologic malignancies, with an estimated 20,000 new MM diagnoses in 2007, and about 11,000 projected to die of the disease this year. The median survival of patients with high-

risk myeloma is <2–3 years compared with a median survival of 6–7 years for patients with standard-risk MM.[2] Several independent prognostic factors have been developed to stratify standard-risk and high-risk myeloma patients in order to estimate median survival and justify treatment approaches. High-risk prognostic factors can be determined by molecular classifications such as deletion 13, deletion 17p, and immunoglobulin-heavy chain translocations t (4; 14) or t (14; 16). Despite the introduction of promising novel agents including thalidomide, bortezomib, and lenalidomide, patients with high-risk myeloma continue to do poorly, even with tandem autologous bone marrow transplantation.[2-3] Multiple previous studies have demonstrated a median progression-free survival of only 7-8 months in patients with high-risk cytogenetics after high-dose therapy and autologous stem cell transplantation.[4-6] This translates into progression-free survival of 35% at one year of this patient cohort.

3.2. Hematopoietic Stem cell Transplantation for Multiple Myeloma

Conventional treatments with chemotherapy and radiation therapy for patients with multiple myeloma (MM) are non-curative but improve quality of life and duration of survival. High-dose chemotherapy with autologous transplantation is safe and has low TRM (< 5%), but is associated with a continuing and nearly universal risk of disease progression and relapse. Even so, autologous transplantation is superior to continued conventional chemotherapy.[7] Although recent data indicate that tandem autologous transplants are superior to a single procedure,[8] even with this approach, patients remain at risk of relapse and additional approaches are needed.

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment available for patients with multiple myeloma. However, at this time, allogeneic hematopoietic stem cell transplantations are not standard of care for multiple myeloma patients as the success rate in these patients has been compromised by a high incidence of transplant-related mortality and high incidence of acute and/or chronic graft-versus- host disease (GvHD).[9-10] This protocol uses T-cell depleted allogeneic stem cell infusions to reduce the risk of transplant-related mortality and graft versus host disease.

3.2.1. Autologous Hematopoietic Stem cell Transplantation for Multiple Myeloma

Barlogie et al[11- 13] demonstrated that the toxicity of high-dose melphalan could be decreased by autologous stem cell transplantation and that the use of peripheral blood hematopoietic stem cells led to wider application of high-dose therapy using autologous stem cell support. With autologous hematopoietic stem cell support following high-dose melphalan, the toxic death rate was less than 3% in most studies[14-15]. At a dose of 200 mg/m^2, high response rates in both untreated and previously treated patients are reported.[16-21] In addition, recent data indicate melphalan 200 mg/m^2 is less toxic but equally effective as melphalan with TBI.[22- 23] Barlogie et al also demonstrated the feasibility of tandem autologous PBSC transplants using melphalan 200 mg/m^2 for the first transplant and melphalan 200 mg/m^2 (79 patients) or melphalan 140 mg/m^2 plus TBI (1,125 cGy) (10 patients) for the second transplant. Of 123 patients, 87% completed one autologous transplant and 76% completed a tandem second autologous transplantation by 7.5 and 13 months, respectively, with a median interval between autologous transplantations of 4.5 months. The group reported 40% CR to tandem transplants and a median event-free survival of 49 months, based on intent to treat analysis in this single center experience. For responding patients, high-dose therapy followed by autologous PBSC rescue has been shown to be superior to continued treatment with conventional chemotherapy, leading to the

widespread use of autologous transplantation for myeloma patients up to and beyond the age of 70.[24-25] In the IFM study, the progression free and overall survival curves separated after 3 years with improved disease free and overall survival at 6 years post-transplantation for the group that received tandem autologous transplants compared to those receiving single transplants. Overall the median event free survival was 31 versus 37 months, 6-year event free survival was 19% versus 28% and 6-year overall survival 26% versus 46% for single versus tandem transplants, respectively. While TRM rates observed in these studies are low, patients continued to remain at risk for disease relapse and progression, with only a minority of patients remaining disease free at six years. Importantly, when Rajkumar et al assessed progression-free and overall survival based on risk category of standard versus high-risk patients, progression-free and overall survival were only 8 months and 14 months, respectively, in patients with high-risk disease who did not achieve a CR following high-dose Melphalan and autologous stem cell transplantation. Patients in the high-risk category achieving a CR following ASCT had a progression-free and overall survival of only 12 months and 23 months, respectively.[2, 7]

3.2.2. Allogeneic Hematopoietic Stem cell Transplantation for Multiple Myeloma

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment available for patients with multiple myeloma. Despite the potential advantages of graft-versus-tumor immune responses and a tumor free source of stem cells, conventional high-dose conditioning with allogeneic bone marrow or PBSC transplantation, the success rate in these patients has been compromised by a high incidence of transplant-related mortality and high incidence of acute and/or chronic graft-versus- host disease (GvHD) and associated complications with transplant-related mortalities (TRM) exceeding 40%.[9-10] The TRM associated with allogeneic hematopoietic stem cell transplantation for MM exceeds that reported after allotransplantation for hematologic malignancies such as acute and chronic myeloid leukemias for unclear reasons. It possibly reflects the high incidence of co morbid disease, especially renal failure. The largest group of myeloma patients treated with allogeneic bone marrow transplantation, reported by Gahrton et al, [9-10] included 162 patients treated in 35 different European centers from 1983 to 1993. Early toxicity was high with an approximate 40% mortality within the first six months. Bensinger et al[7] reported on 80 patients treated between 1987 and 1994. Seventy-one percent of these patients were considered to have refractory disease at the time of transplant. TRM was high, with 42% deaths within the first 100 days and a few additional treatment-related deaths later. When used, it is generally limited to younger patients, in contrast to the advanced age profile of MM patients at diagnosis. In the experience at Fred Hutchinson Cancer Research Center (FHCRC), Day 100 TRM after conventional allografting exceeded 50% in patients ≥ 50 years of age. The inability of myeloma patients to tolerate allografting remains largely unknown, but may relate to an inability of generally elderly and immune suppressed patients to tolerate the combined effects of high-dose therapy and allografting in a single procedure. In contrast, a Phase II study using a two-step approach where high-dose melphalan and autologous stem cell transplantation was followed by a non-myeloablative allogeneic transplant showed that donor engraftment can be established with a conditioning regiment of low dose total body irradiation (TBI) (200 cGy), cyclosporine (CSA) and mycophenolate mofetil (MMF) combined with peripheral blood stem cell (PBSC) allografting. This study demonstrated that allografting can be safely and effectively performed if the high-dose regimen is administered separate from the allografting procedure. In this study, approximately 60% of the patients

achieved complete remission (CR) with an overall-survival of approximately 85% at one year.[26] One of the objectives of the study to reduce TRM at three months to < 20%, was achieved, as only 13% of patients experienced treatment-related mortality.[26-27] Overall, the introduction of non-myeloablative for conditioning therapies in the treatment of myeloma has demonstrated reduced toxicity and transplant-related mortality.[13-16, 20] However, results from transplants with non-myeloablative regimens were poor if the patient had failed prior autologous bone marrow transplantations or had developed chemotherapy-resistant disease.[17, 20]

To extend the potential benefits of allogeneic transplants to the full range of patients developing disorders for which a transplant is indicated, particularly older patients (> 50 years of age), who constitute the majority of patients with MM, as well as patients lacking HLA-matched sibling donors, major emphasis has been focused on the development of approaches which could circumvent severe acute and chronic GvHD so as to reduce its morbidity and mortality in both HLA-matched and HLA-non-identical transplant recipients. While no combination of immunosuppressive drugs administered to recipients of HLA-matched related or unrelated unmodified hematopoietic stem cells grafts has prevented the development of acute and chronic GvHD, this serious transplant related complication can be prevented by the depletion of T lymphocytes from the allograft prior to administration in HLA matched related and unrelated recipients as well as in HLA-disparate recipients.[28-35] In our own series, among 232 consecutive adult leukemic patients (median age 41) engrafted with marrow transplants from HLA-matched siblings depleted of T-cells by soy bean agglutinin and E rosette depletion, the incidence of grade II-III GvHD was 3% and the incidence of chronic graft vs. host disease was 5%.[36] Similarly, in over 100 unrelated marrow grafts transplanted using this T-cell depletion technique, the incidence of grade II-IV GvHD and of chronic GvHD has been 8%.[37]

Initially, the central limitation to the effectiveness of T-cell depleted marrow transplants was a high risk for graft rejections or late graft failures. Studies conducted at our institution demonstrated that such graft failures are principally caused by residual host T-lymphocytes which regenerate early after transplant and are able to reject donor hematopoietic cells through their cytotoxic interactions with major class I or class II HLA alloantigens in recipients of HLA-disparate transplants, or minor alloantigens presented by HLA class I determinants in recipients of HLA-matched grafts.[38-39] Based on these findings, our group conducted a sequence of trials combining myeloablative doses of either total body irradiation, thiotepa and fludarabine or busulfan, melphalan and fludarabine with antithymocyte globulin (ATG) which reduced the incidence of graft failures following HLA-matched T cell depleted transplants to less than 2% and the incidence of graft failure following unrelated and 1-2 allele disparate marrow grafts to less than 8%.[36-37, 40] These rates of graft failure are comparable to those observed following unmodified transplants from such donors.[41] Additionally, Aversa et al[42-44] demonstrated that when G-CSF mobilized peripheral blood stem cells are utilized as the source of hematopoietic stem cells for T-cell depleted graft, the doses of stem cells provided are 5-10 fold higher than those that can be achieved in a marrow transplant and allow a durable engraftment and hematopoietic reconstitution even in HLA-haplotype disparate leukemic recipients.[42] Based upon this observation, G-CSF mobilized peripheral blood stem cells are currently the preferred source of hematopoietic cells for T cell depleted transplants.

3.2.3. Donor Lymphocyte Infusions

Initial proof of donor lymphocytes derived from the same hematopoietic stem cell donor to exert a graft-versus-leukemia effect resulted from the pioneering findings of Kolb et al[45] and was later confirmed by several centers.[46-50] Patients who developed relapses of chronic myelogenous leukemia (CML) following an allogeneic marrow transplant were induced into durable molecular remissions by high doses of peripheral blood mononuclear cells derived from the original HLA-matched transplant. These studies provided the first evidence that enhanced resistance to leukemia, accrued through a marrow allograft by comparisons of relapse rates following syngeneic vs HLA-matched allogeneic transplants, was indeed mediated by cells in the donor graft.[46]

Several observations suggest the existence of graft-versus-myeloma immune activity following allogeneic transplantation. Bjorkstrand demonstrated indirect evidence in a case-matched analysis of 189 myeloma patients treated with either autologous transplant or allogeneic transplantation. He found a significantly higher rate of relapse from CR or disease progression from PR in the autologous transplant group compared to patients who underwent allogeneic transplantation.[51] In addition, the quality of remission appeared greater following allogeneic transplantation as molecular remissions were rarely (7%) achieved after autografting (n=15) compared to a 50% molecular remission rate seen after allografting (n=14).[52] These data suggest that the chemoresistance of myeloma cells might be overcome by immune antitumor effects of the allograft. The existence of a graft-versus-myeloma effect has been more directly confirmed by results of donor lymphocyte infusion (DLI) in patients who relapse after failure of conventional allografts.[53-55] Salama reported results on 25 patients who received DLI (median dose $1x10^8$ mononuclear cells/kg) for MM relapsing after an allograft. Some patients received chemotherapy prior to the DLI and some received multiple escalating doses of DLI. Overall, 7 of 15 achieved a CR and 3, a partial response (PR). Lokhorst[53] reported on 27 patients receiving DLI following partially T-cell depleted allo transplants. The DLI were given a median of 30 months post-transplant. Overall, 14 of 27 patients responded with 5 CRs. Responding patients received at least $1x10^8$ mononuclear cells/kg. All responding patients developed graft-versus-host disease following administration of the relatively high doses of donor T lymphocytes.

Alyea et al. combined a CD6 T-cell-depleted allogeneic BM with prophylactic administration of $CD4^+$ DLI 6 to 9 months after BMT in an effort to reduce TRM and to induce a GVM response after BMT. Twenty-four patients with matched sibling donors and chemotherapy-sensitive disease underwent BMT following a TBI, Cytoxan or Cytoxan, Busulfan conditioning regimen. CD6 T-cell depletion of donor bone marrow was the sole method of graft-versus-host disease (GvHD) prophylaxis. GvHD after BMT was minimal, 1 (4%) grade III and 4 (17%) grade II GvHD. Fourteen patients received DLI ($1\text{-}3x10^7$/kg $CD4^+$ cells), 3 in complete response and 11 with persistent disease after BMT. Significant GVM responses were noted after DLI in 10 patients with persistent disease, resulting in 6 complete responses and 4 partial responses. After DLI, 50% of patients developed acute (> or = II) or extensive chronic GvHD. Two-year estimated overall survival and current progression-free survival (PFS) for all 24 patients is 55% and 42%, respectively. The 14 patients receiving DLI had an improved 2-year current PFS (65%) when compared with a historical cohort of MM patients who underwent CD6-depleted BMT survived 6 months with no GvHD and did not receive DLI (41%) (P =.13). Although this study suggested that prophylactic DLI induced significant GVM responses after allogeneic BMT, only 58% of patients

were able to receive DLI despite T-cell depleted BMT due to transplantation associated toxicities. The authors of this study therefore suggested that less toxic transplantation strategies are needed to allow a higher proportion of patients to receive DLI and the benefit from the GVM effect after transplantation.[56]

3.3. Rationale for Study

This study aims at improving progression-free survival while reducing the transplantation-related complications in patients with relapsed or high-risk multiple myeloma. To achieve these goals, we propose to perform T-cell depleted allogeneic hematopoietic stem cell transplantation from related or unrelated matched or one HLA-antigen/allele mismatched donors with chemotherapy only conditioning and prophylactic/preemptive administrations of calculated doses of donor lymphocyte infusions, derived from the same hematopoietic stem cell donor. Our initial experience with T-cell depleted transplants has been in patients with myelodysplastic syndrome and acute leukemia. Our results summarized below showed that patients with advanced MDS have better transplant outcomes if they are transplanted in remission after chemotherapy. Because MDS patients are mostly older patients (>age 40), as are patients with multiple myeloma, and some of them are not eligible to receive total body irradiation, our institution developed a chemotherapy only preparative regimen including busulfan, melphalan, fludarabine and anti-thymocyte globulin for these patients who could also benefit from T-cell depleted SCT from HLA matched or HLA disparate related and unrelated donors. The results of our experience of T-cell depleted SCT in patients with advanced MDS with the chemotherapy only preparative regimen are summarized in the next paragraph.

A phase II trial with a chemotherapy only conditioning was performed from August 2001 – June 2007 (Boulad et al ASH 2007). This trial demonstrated that the use of busulfan, melphalan and fludarabine followed by T-cell depleted transplants from matched related, mismatched related, matched unrelated or mismatched unrelated donors in patients with advanced MDS is safe and has a significant efficacy as demonstrated by the improved DFS. This trial included 63 patients; 7 children and 56 adults. The median age was 55.4 years with a range of 0.6 to 71.3; forty pts were 50 with 12 pts older than 60 years. The diagnoses included: primary MDS (33 patients) and AML evolved from MDS (20 patients), de novo AML in CR1 (9 patients) or CR 2 (1 patient). The donors were HLA matched (A, B, C, DR, DQ by high resolution) related (13), mismatched related (4), matched unrelated (46). Except in two patients, the source of stem cells was peripheral blood. Sixty two patients were evaluable for outcomes and had at least 6 months of post transplant followup. Results of this trial demonstrated that this preparative regimen was safe as determined by the low incidence of graft rejection, and GvHD. All but 2 patients achieved durable engraftment. One recipient of unrelated HLA disparate transplant experienced primary graft failure and one of unrelated HLA disparate transplant experienced graft rejection. Acute GvHD II- IV developed in 7 patients (18%) and chronic GvHD in 4 patients (7%) (3 extensive), despite the advanced age of this group. Moreover, oral mucositis was less severe as compared to the mucositis seen after myeloablative doses of TBI-containing regimens. Thus, the objectives of securing consistent engraftment with graft failure <10% and grade II-IV acute GvHD in 1 HLA disparate locus recipient have been met in this trial.

The prophylactic/preemptive administration of calculated doses of donor lymphocyte infusions has been integrated to reduce potential relapses of the disease. Historically, donor

lymphocyte infusion were performed at time of relapsed disease, requiring doses of $1x10^7$ - $1x10^8$/kg $CD3^+$ cells to induce a remission. As detailed above under *3.2.3. Donor Lymphocyte Infusions,* these doses have also been associated with development of acute and chronic graft-versus-host disease. As proposed in this trial, doses of donor-derived $CD3^+$ T-cells will be given in the range of $5x10^5$/kg $CD3^+$ cells from matched donors and $1x10^5$/kg $CD3^+$ cells from one allele/antigen mismatched donors with a first dose administered 5-6 months post transplantation. Finally, the addition of ATG to the chemotherapy only conditioning regim en with busulfan, melphalan and fludarabine in our previous trial has shown to reduce the incidence of graft failures following HLA-matched T-cell depleted transplants to less than 2% and the incidence of graft failure following unrelated with 1-2 allele disparate marrow grafts to less than 8%.

Pilot trial

To assess whether this chemotherapy only preparative regimen including busulfan, melphalan, fludarabine and anti-thymocyte globulin could be safely administered to patients with relapsed high-risk multiple myeloma and whether these patients who could benefit from T-cell depleted allogeneic hematopoietic stem cell transplantation from HLA matched related or unrelated donor, we performed an initial pilot trial. Five patients with high-risk MM, all of which relapsed following multiple chemotherapy regimen and after one or two preceding autologous stem cell transplantations underwent T-cell depleted transplants from HLA-matched siblings (3pts), matched unrelated (1pt) or mismatched unrelated (1pt) donors. Details of the individual patients and their outcomes are described in the paragraph below, but in summary, all five patients durably engrafted with 100% donor chimerism with patients being 22, 16, 14, 10 and 4 months following the allogeneic stem cell transplantation. None of the patients developed acute, chronic graft-versus-host disease or graft failure at this time. Three of these patients achieved a complete remission, one patient remains in near complete remission and one patient had stable disease with M-spike of 0.2g/dl for 20 months following the transplant.

UPN #1 is a 40+ y/o male, diagnosed with stage III IgG kappa MM. He was treated with Thalidomide and Dexamethasone followed by his 1^{st} autologous SCT with Mel 200 and 2^{nd} autologous SCT 4 months later. He relapsed after 15 months and was treated with Lenalidomide and Dexamethasone x 3 cycles. He underwent allogeneic HSCT from 9/10 unrelated female with HLA-DQB1 antigen mismatch 22 months post autologous SCT. The patient had 7% Plasma cells in his marrow at admission. The patient tolerated the transplantation very well, engrafted promptly without requiring readmission following discharge from this hospitalization. The patient received infusion of low doses of donor lymphocytes ($5x10^4$/kg) at 5 months and 12 months post transplant and remained at very stable disease with low levels of M-protein ranging between 0.2 and 0.3 mg/d x 20 months. This patient has recently developed signs of progression of disease and received $1x10^6$/kg $CD3^+$ donor T cell infusion two months ago and it is currently too early to assess the response, but he has not developed signs of GvHD.

UPN #2 is a 30+ y/o male, who was diagnosed with stage III IgA lambda with high-risk cytogenetics, (del 13, t (4; 14). He was treated with Velcade and Dexamethasone x 6 cycles followed by an autologous SCT. He relapsed 3 months later. The patient was given 1 cycle of Lenalidomide and Dexamethasone, which he tolerated very poorly and was switched to Velcade and Dexamethasone x 2 cycles. He underwent allogeneic HSCT from his 10/10 identical sister in

9 months post autologous SCT. He is currently 16 months post transplant in complete remission without complaints. The patient received two doses of 5x 10^5/kg $CD3^+$ donor lymphocytes at 7 and 14 months without signs of acute or chronic graft-versus-host disease.

UPN #3 is a 30+ y/o male with stage III IgG kappa MM diagnosed with deletion 13. He was initially treated with Lenalidomide and Dexamethasone x 5 cycles without a response. He was then treated with VAD x 5 cycles with only limited response (28% plasma cells in marrow), and continued with autologous SCT with minimal response. He was given Velcade/Dexamethasone and Thalidomide x 4 cycles with partial response. He underwent allogeneic HSCT from his 10/10 identical sister 7 months post autologous SCT. The patient tolerated the transplantation without complications, stably engrafted with 100% donor chimerism and is back to work fulltime. He is currently 14 months post transplant in near complete remission. The patient received two doses of 5x 10^5/kg $CD3^+$ donor lymphocytes at 6 and 10 months without signs of acute or chronic graft-versus-host disease.

UPN #4 is 50+ y/o male, diagnosed with stage III IgG lambda MM with high-risk cytogenetics (del 17p, del 13q, t11; 14). He was treated with Thalidomide and Dexamethasone x 4 months with progression of disease + renal failure, followed by Velcade, interrupted by development of myocardial infarction followed by CAGB in the setting of CHF, EF 35%. Afterwards he was continued on Velcade x 1 cycle followed by VP-16 + Cytoxan with PR. He underwent autologous SCT with Melphalan 200 and relapsed after one year. He was given VAD with PD and VP-16/Cytoxan x 3 cycles with partial response. He underwent allogeneic SCT from 10/10 unrelated donor 17 months post autologous SCT and engrafted promptly without complications. The patient is currently 10 months post transplant with all donor chimerism and in complete remission. The patient received a first dose of 5x 10^5/kg $CD3^+$ donor lymphocytes at 6 months and has currently no signs of acute or chronic graft-versus-host disease.

UPN #5 is a 60+ y/o male, diagnosed with stage III IgG kappa MM. He was initially treated with Revlimid, Dexamethasone and /Biaxin (BIRD) with PR. Autologous SCT was performed with good response, but the patient relapsed after one year. He was started on Revlimid, Velcade and Dexamethasone x 4 cycles followed by Revlimid maintenance therapy, which he tolerated poorly. Allogeneic SCT from 10/10 HLA matched brother was performed 2 years post autologous SCT. This patient engrafted promptly with all donor chimerism and achieved complete remission post transplantation, but unfortunately passed away in 5 months post allogeneic SCT with respiratory failure as a consequence of pneumonia. The cause of the pneumonia was likely viral, but the patient had refused to be intubated and a bronchoscopy could not be performed.

These results can be compared to the outcome of patients enrolled by our group onto the national BMT CTN trial #04-024. On this protocol, all patients with multiple myeloma, independently of risk category of cytogenetics of their disease, who had an HLA-identical sibling, were treated with an autologous stem cell transplant with Melphalan 200mg/m^2 conditioning followed by a non-myeloablative allogeneic transplant from the sibling with 200cGy Total Body Irradiation as conditioning regimen. The complete and official analyses of this national trial are still pending, but there were 9 patients enrolled onto the auto/allo arm of this trial by our group. Seven of these 9 pts actually underwent allogeneic transplantation. Of these 7 pts, 3 pts developed acute graft-versus-host disease, of which 2 pts also developed chronic GvHD. 2 of 7 pts

developed chronic GvHD without preceding acute GvHD. Two of 7 patients died. One patient died 7 months following the transplant secondary to complications of acute GvHD, the other patient died of progression of disease.

3.5. Summary

The success of allogeneic SCT in patients with relapsed or high-risk multiple myeloma depends on multiple variables. Our approach involving T-cell depleted, reduced-intensity, myeloablative allogeneic transplant has the potential to overcome two major obstacles limiting the success of allogeneic transplantation for patients with multiple myeloma, namely graft-versus-host disease and associated morbidity and mortality. The prophylactic/preemptive administration of low doses of donor lymphocyte infusions is designed to reduce the relapse mortality post transplant and to improve the PFS with this treatment. In the pilot trial of 5 patients treated with high-risk multiple myeloma, as described in detail above, this approach has been well tolerated in these patients with high-risk and relapsed MM. None of these patients has development of graft-versus-host disease or graft failure. Furthermore, 3 of the 5 patients transplanted achieved a complete remission and 2 patients obtained near complete remissions with lasting durations.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a two arm phase II trial to assess overall survival, progression-free survival, efficacy (decrease the transplant related mortality) and safety of allogeneic hematopoietic stem cell transplantation using a preparative regimen with busulfan, melphalan, fludarabine, and anti-thymocyte globulin (ATG), and T-cell depleted stem cell transplant from a histocompatible related or unrelated donor in patients with relapsed or high-risk multiple myeloma. The prophylactic or preemptive administration of donor lymphocyte infusions is performed to decrease the rate of relapse and thus to prolong event-free and overall survival.

4.3 Intervention

Patients will be cytoreduced with IV busulfan, melphalan and fludarabine. Busulfan will be administered at a dose of 0.8mg/Kg q6hrs for 10 doses on days -9, -8, -7. Doses for busulfan should be adjusted according to pharmacokinetic studies. Melphalan will be administered at a dose of 70 mg/m^2/day for 2 days on days -7, -6. Fludarabine will be administered at a dose of 25 mg/m^2/day for 5 days on days -6, -5, -4, -3, -2. Patients will also receive rabbit anti-thymocyte globulin (ATG) to prevent immune mediated graft rejection. The ATG will be administered at a dose of 2.5 mg/Kg/day IV on days -3 and -2. Doses for busulfan, melphalan and ATG should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight. Doses of fludarabine should be reduced to 80% of dose for measured creatinine clearance of 40-70ml/min. Day -1 will be a day of rest before receiving the T-cell depleted stem cell product on day 0. The preferred stem cell product will be peripheral blood stem cells but if the donor is unable or unwilling to donate peripheral blood stem cells, bone marrow will be used as the stem cell source. Patients will receive post-transplant G-CSF starting on day +7.

Historically, under the multicenter BMTCTN trial 0303 (IRB 06-005) which employed the CliniMACS device, GCSF mobilized PBSC, after washing, were suspended in buffered saline only and then incubated with antiCD34 coated paramagnetic beads prior to separation of the $CD34^+$ cells bound to the beads by adherence to an electromagnet in the CliniMACS device. A provision recommended as an adjunct but not required or known to be necessary was that the diluent in which the washed GCSF-mobilized PBMC were incubated with the antiCD34 coated beads also include 30% autologous plasma or intravenous gamma globulin at a concentration of 1.5 mg/ml. This provision was not used by the centers participating in the BMTCTN trial, including our own. That trial recorded an incidence of acute grade II-IV GVHD of 20.5% and a 7.6% incidence of extensive GVHD. While these findings were significantly better than any results recorded with drug prophylaxis or methods of T-cell depletion developed at other centers, the incidence of acute GVHD was higher than what we had published using SBA^-E^- T-cell depleted marrow or $CD34^+$ (ISOLEX) E^- T-cell depleted PBSC. Since we did not see significant GVHD in the small number of patients that we contributed to the trial, we ascribed the higher incidence to limited experiences with the CliniMACS device and to recent changes in the grading system used for acute GVHD. Accordingly, no changes in procedures were made to protocol 10-051.

However, as accrual to 10-051 proceeded, we observed an increase in GVHD even in HLA-matched cases, and severe acute GVHD in some patients who received partially HLA-matched transplants. Again, the data were not different from the BMT CTN trial results, but these results were different compared to our experience with patients previously transplanted with T-cell depletion by the ISOLEX method. However, the rate of acute GVHD was higher than that reported by 2 European groups using the CliniMACS device for HLA disparate grafts. We subsequently learned that the largest of these centers, Perugia U., did incorporate IVIG in the incubation step.

Considering the possibility that in the absence of plasma or IVIG, cells can non-specifically adsorb to paramagnetic beads, the cytotherapy laboratory validated separation of $CD34^+$ cells on the CliniMACS in the presence or absence of IVIG in the incubation period, demonstrating comparable yields of $CD34^+$ cells and similar levels of T-cell depletion. Thereafter, on 7/25/12, the procedure was modified to include the IVIG. In February 2013, we evaluated the incidence of acute grade II-IV GVHD and chronic GVHD in patients treated since then. The incidence of acute grade II-IV GVHD has been significantly lower approximating 5%.

In order to be able to meaningfully compare the incidence of acute GVHD in patients receiving transplants fractionated in the presence or absence of IVIG and therefore obtain a better overall understanding of the actual rate of acute GvHD, we wish to continue to enroll patients on the relapsed arm and increase the number of pts by 17 pts to a total of 47 pts.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1. *Busulfan (Busulfex®)*

a. Source and pharmacology: Supplier: Otsuka Pharmaceutical; Busulfan is a bifunctional alkylating agent known chemically as 1, 4-butanediol, dimethanesulfonate. BUSULFEX® (busulfan). This is an agent in which two labile methanesulfonate groups are attached to opposite ends of a four carbon alkyl chain. In aqueous media, busulfan hydrolyzes to release the methanesulfonate groups. This produces reactive carbonium ions that can alkylate DNA. DNA damage is thought to be responsible for much of the cytotoxicity of busulfan.

b. Formulation and stability: It is supplied as a clear, colorless, sterile, solution in 10 mL single use ampoules. Each ampoule of BUSULFEX contains 60 mg (6 mg/mL) of busulfan, the active ingredient, a white crystalline powder with a molecular formula of CH_3SO_2O $(CH_2)_4OSO_2CH_3$ and a molecular weight of 246 g/mole. Busulfan is dissolved in N, N-dimethylacetamide (DMA) 33% wt/wt and polyethylene glycol 400, 67% wt/wt. Busulfan‟s solubility in water is 0.1 g/L and the pH of a >0.5% solution in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP as recommended for infusion reflects the pH of the diluent used and ranges from 3.4 to 3.9.

c. Solution preparation: BUSULFEX is supplied as a sterile solution in 10 mL single-use clear glass ampoules each containing 60 mg of busulfan at a concentration of 6 mg/mL for intravenous use. BUSULFEX must be diluted prior to use with either 0.9% Sodium Chloride Injection, USP (normal saline) or 5% Dextrose Injection, USP (D5W). The diluent quantity should be 10 times the volume of BUSULFEX, ensuring that the final concentration of busulfan is approximately 0.5 mg/mL

d. Storage and stability: Unopened ampules of BUSULFEX must be stored under refrigerated conditions between 2° -8° C (36° -46° F).

e. Administration: Intravenous, over 2 hours.

5.2. *Melphalan (Alkeran®)*

a. Source and pharmacology: Supplier: Glaxo Wellcome. A derivative of nitrogen mustard, an analog of mustard gas. It is a polyfunctional alkylating agent that causes miscoding, cross-linkage of DNA, and single-strand breakage of DNA. It inhibits cellular glycolysis, respiration, and protein synthesis. It is cell cycle-phase non-specific.

b. Formulation and stability: A lyophilized powder of 50 mg melphalan and 20 mg povidone per vial. Also provided is 10 ml of sterile diluent for use in reconstituting the product and a 0.45 micron filter. The special diluent has the following composition: Sodium citrate 0.2 g, Propylene glycol 6.0 ml, Ethanol (95%) 0.5 ml, and sterile water 10 ml.

c. Solution preparation: Vial/50 mg: Reconstitute by rapidly injecting 10 ml of the supplied diluent into the vial to yield a final concentration of 5 mg/ml. Shake vigorously until the solution is clear. Immediately dilute the dose to be administered in 0.9% Sodium Chloride, USP, to a concentration no greater than 0.45 mg/ml

d. Storage and stability: The intact packages should be stored at room temperature (15-30°C) protected from light. Shelf-life surveillance of the intact dosage form is ongoing. Constitution with the special diluent as directed results in a solution that retains at least 90% potency for about three hours at 30°C. Storage at 5°C results in precipitation.

e. Administration: Intravenous, over 30 minutes. Complete infusion within 60 minutes of preparation.

5.3 *Fludarabine (FLUDARA®)*

a. Source and pharmacology: Supplier: Berlex Laboratories, Inc. FLUDARA FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-ß-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-ß-D-arabinofuranosyl). Fludarabine phosphate 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.

b. Formulation and stability: Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration. FLUDARA FOR INJECTION is supplied in a clear glass single dose vial (6 mL capacity) and packaged in a single dose vial carton in a shelf pack of five

c. Solution preparation: FLUDARA should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. 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

d. Storage and stability: FLUDARA is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2°-8° C (36°-46° F).

e. Administration: Intravenous, over thirty minutes.

5.4. *Anti-Thymocyte Globulin (Rabbit) (Thymoglobulin®)*

a. Source and pharmacology: Supplier: Sangstat, The Transplant Company®. Thymoglobulin® [Anti-thymocyte Globulin (Rabbit)] is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes.

b. Formulation and stability: Thymoglobulin is a sterile, freeze-dried product for intravenous administration after reconstitution with sterile Water for Injection, USP (WFI). Each package contains two 7 mL vials: Vial 1: Freeze-Dried Thymoglobulin Formulation Active ingredient: Anti-thymocyte Globulin (Rabbit) 25 mg - Inactive ingredients: Glycine (50 mg), mannitol (50 mg), sodium chloride (10 mg); Vial 2: Diluent Sterile Water for

Injection, USP 5 mL. The reconstituted preparation contains approximately 5 mg/mL of Thymoglobulin, of which >90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7.0± 0.4. Human red blood cells are used in the manufacturing process to deplete cross-reactive antibodies to non-T-cell antigens. The manufacturing process is validated to remove or inactivate potential exogenous viruses. All human red blood cells are from US registered or FDA licensed blood banks. A viral inactivation step (pasteurization, i.e., heat treatment of active ingredient at 60°C/10 hr) is performed for each lot. Each Thymoglobulin lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, antiglomerular basement membrane antibody, and fibroblast toxicity assays on every 5th lot).

c. Solution preparation: Each reconstituted vial contains 25 mg or 5 mg/mL of Thymoglobulin. Transfer the contents of the calculated number of Thymoglobulin vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of Thymoglobulin use 50 mL of infusion solution (total volume usually between 50 to 500 mL). Mix the solution by inverting the bag gently only once or twice.

d. Storage and stability: Store in refrigerator between +2° C to +8° C (36° F to 46° F). Protect from light. Do not freeze. Do not use after the expiration date indicated on the label. Reconstituted vials of Thymoglobulin should be used within 4 hours. Infusion solutions of Thymoglobulin must be used immediately. Any unused drug remaining after infusion must be discarded.

e. Administration: Infuse through a 0.22-micron filter. Set the flow rate to deliver the dose over 12 hours.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

<u>Diagnosis</u>:

Patient must have multiple myeloma that has either relapsed or has high risk cytogenetics.

1. Patients with relapsed multiple myeloma following autologous stem cell transplantation must have achieved at least partial response following additional chemotherapy (cohort 1):
a. Patients are eligible if relapse occurs with complex/high-risk cytogenetics or occurs with normal cytogenetics but within 15 months following the autologous transplant.

2. Patients with high risk cytogenetics at diagnosis must have achieved at least very good partial response following autologous stem cell transplantation (cohort 2):
a. Patients must have complex karyotype, 1q25, del17p, t4;14 and/or t14;16 by FISH and/or del13 by karyotyping.

DONOR: Patients must have a healthy HLA matched or mismatched related or unrelated donor who is willing to receive G-CSF injections and undergo apheresis for PBSC collection, or undergo a marrow harvesting procedure.

1. HLA-matched related and unrelated donors

Patients who have an HLA-matched related or unrelated donor are eligible for entry on this protocol. This will include a healthy donor who is genotypically matched at all A, B, C, DRB1 and DQB1 loci, as tested by DNA analysis.

2. HLA- mismatched related and unrelated donors

Patients who do not have an HLA-matched donor but have a related or unrelated donor who have one antigen or one allele mismatch at the HLA A, B, C, DRB1 or DQB1 loci; or who have two mismatches, at HLA-DQB1 and at one other locus, will be eligible for entry on this protocol.

The following inclusion criteria are also required:

- Patients should be ≥ 21, < 73 years old.
- Patients may be of either gender or any ethnic background.
- Patients must have a Karnofsky (adult) or Performance Status ≥ 70%
- Patients must have adequate organ function measured by:

a) *Cardiac*: asymptomatic or if symptomatic then LVEF at rest must be ≥ 50% and must improve with exercise.

b) *Hepatic:* < 3x ULN ALT and < 1.5 total serum bilirubin, unless there is congenital benign hyperbilirubinemia.

c) *Renal:* serum creatinine ≤1.2 mg/dl or if serum creatinine is outside the normal range, then CrCl > 40 ml/min (measured or calculated/estimated) with dose adjustment of Fludarabine for <70ml/min.

d) *Pulmonary:* asymptomatic or if symptomatic, DLCO > 50% of predicted (corrected for hemoglobin)

- Each patient must be willing to participate as a research subject and must sign an informed consent form.

6.3 Subject Exclusion Criteria

- Patients achieving < Partial Response following preceding chemotherapy (cohort 1) or < Very Good Partial Response following autologous stem cell transplantation (cohort 2).
- Patients with Plasma Cell Leukemia.
- Female patients who are pregnant or breast-feeding
- Active viral, bacterial or fungal infection
- Patient seropositive for HIV-I/II; HTLV -I/II
- Patients who have undergone prior allogeneic hematopoietic stem cell transplantation.
- Patients who have had a previous malignancy that is not in remission.
- Patients with known hypersensitivity to mouse proteins (murine antibodies in ISOLEX) if receiving SBA-E- bone marrow, or chicken egg products.

7.0 RECRUITMENT PLAN

Patients with high-risk and relapsed multiple myeloma are referred from collaborating outside institutions and hematology services within MSKCC. All prospective patients are reviewed during the weekly Bone Marrow Transplant meetings. These meetings are attended by the doctors, nurses and members of the research team. Patients who fulfill the eligibility criteria as listed in Section 6.0 will be recruited for this study by Attending Physicians of the Allogeneic BMT service. This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations.

8.1 PRETREATMENT EVALUATION

8.1. **Pretreatment evaluation of the patient**

The patient will receive an extensive medical evaluation within approximately 45 days prior to starting preparatory cytoreduction. This evaluation includes:

- Complete physical exam and medical history
- Dental evaluation (not required within 45 day window)
- CBC
- Coagulation profile
- Blood Type and screen (not required within 45 day window)
- Myeloma specific analyses include – Serum protein electrophoresis (SPEP), Serum Immunofixation (IF), quantitative immunoglobulins, Serum Free Light Chain assay, β-2 microglobulin, LDH; 24 hr urine collection for total protein, creatinine clearance, urine protein electrophoresis (not required if total protein is < 10.0 mg/dl), urine immunofixation.
- Serum chemistries including BUN, creatinine, electrolytes, glucose, total protein, albumin, liver function tests (AST, ALT, bilirubin, and alkaline phosphatase).
- Infectious disease markers will be performed as per each department‟s guidelines or at the discretion of the treating attending.
- Blood will also be tested for HTLV-1 and 2 as well as HIV-1 and 2
- Pregnancy test for women of childbearing age
- Urinalysis,
- Electrocardiogram, echocardiogram or a gated pool scan if needed
- Pulmonary function test
  - Skeletal Survey
  - Chest X-ray (CT scan and PET scan if indicated)
- Samples of bone marrow and/or blood cells will be obtained to assess disease status and to define donor/host differences.
- An additional 3-4 GTTs of blood will be drawn for laboratory correlative studies during routine blood tests.

- Bone marrow assessment will be performed as follows:
  1. Biopsy will be stained with CD138, CD20 and kappa/lambda.
  2. Aspirate will be sent for the following tests:
  a. Routine staining and cell counts
  b. one heparinized syringe with 5ml of marrow for flow cytometry for routine myeloma markers
  c. two syringes with EDTA and 2-4ml of marrow in each syringe for karyotype for chromosomal abnormalities and FISH

9.0 TREATMENT/INTERVENTION PLAN

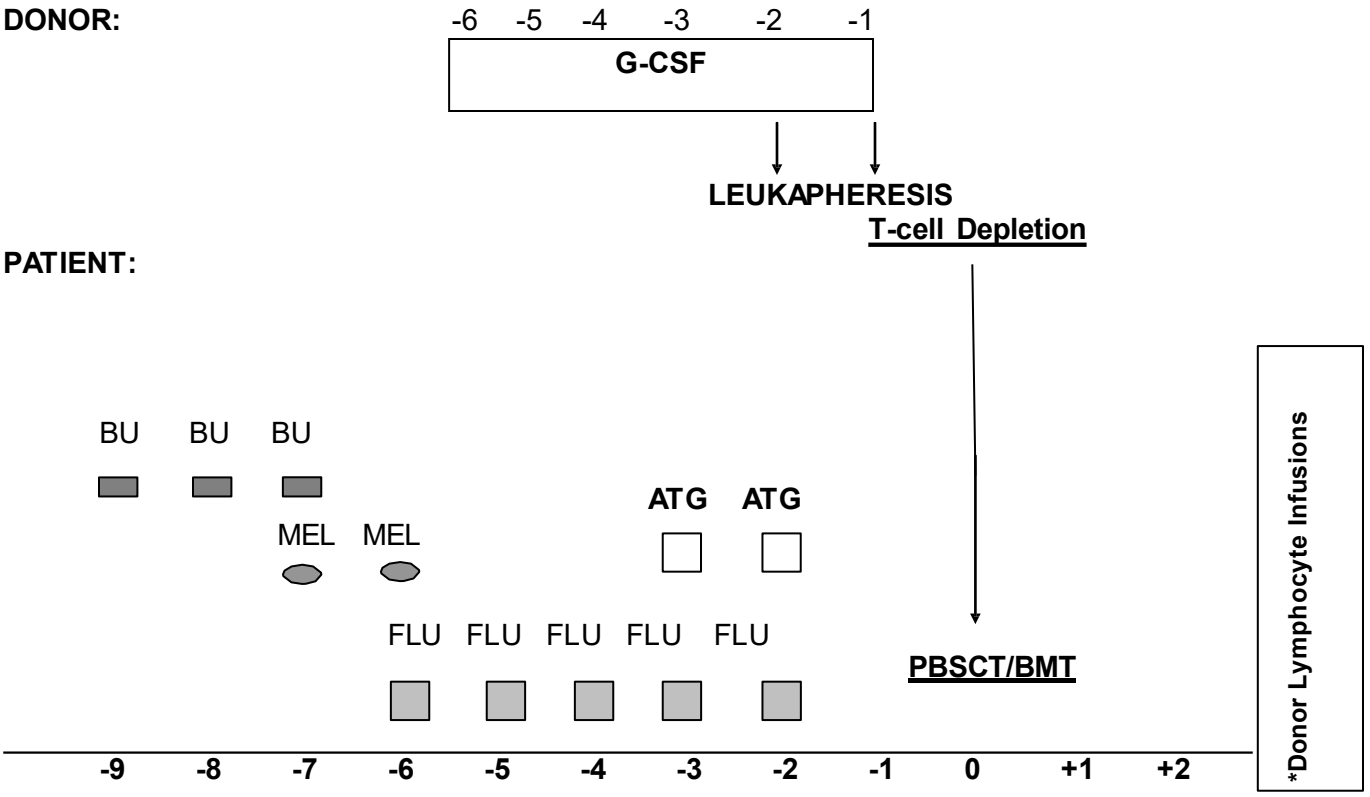
9.1. *Preparative cytoreduction*

The patient will be admitted to a single room on the Adult Transplantation Service. The patient will be maintained in reverse isolation until the day of discharge. Prior to the administration of the pre transplant cytoreductive regimen, a double or triple lumen central venous catheter will be inserted by the Surgical or Interventional Radiology Services.

The preparative cytoreduction will include the following:

Days –9 to –7 **Busulfan* 0.8 mg/Kg/dose Q6H X 10 doses/3 days IV** over 2 hours
Days -7 to -6 **Melphalan** 70 mg/m^2/day x 2 days IV** over 30 minutes
Days -6 to -2 **Fludarabine*** 25 mg/m^2/day x 5 days IV** over 30 minutes

SCHEMA OF CYTOREDUCTION AND PREPARATION FOR ALLOGENEIC PBSCT



*Donor Lymphocyte Infusions –

a. Recipients of HLA-matched allografts will be treated prophylactically with $5x10^5$ $CD3^+$/kg at 4-6 months post transplant. The second infusion of $5x10^5$ $CD3^+$/kg will be administered 3-4 months following the first infusion. A third dose of $1x10^6$ $CD3^+$/kg from matched donors can be administered 2-4 months following the second infusion, if the patient is not in complete remission. All infusions will be administered only in the absence of graft-versus-host disease.

b. Recipients of HLA-mismatched allografts will be treated preemptively with $1x10^5$ $CD3^+$/kg at diagnosis of relapse or progression, but no sooner than 4-6 months post transplant. A second infusion of $5x10^5$ $CD3^+$ cells/kg will be administered 1-3 months following the first infusion. A third infusion of $1x10^6$ $CD3^+$ cells/kg can be administered 3-4

months following the second infusion, if the patient did not achieve a complete remission. Chemotherapy can be given prior to any DLI if it is clinically appropriate. All infusions will be administered only in the absence of graft-versus-host disease.

c. Recipients of HLA-matched allografts will be treated preemptively at diagnosis of relapse or progression and according to the treatment plan described for those recipients of HLA-mismatched allografts.

The below patients will be taken off treatment and treated according to the standard of care. These patients will remain on study and continue to be evaluated according to the protocol:
-Patients who relapse or have progression of disease before they are eligible to receive their DLI.
-Patients who are unable to get 2 of their DLIs as specified by the protocol.

EXAMPLE OF ROAD MAP OF PREPARATION FOR TRANSPLANT

	-10 *Keppra*	-9 Busulfan IV X4 *Keppra*	-8 Busulfan IV X4 *Keppra*	-7 Busulfan IV X2 Melphalan 70 mg/m2/day IV X 1 *Keppra*	-6 Fludarabine 25 mg/m2/day IV X 1 Melphalan 70 mg/m2/day IV X 1 *Keppra* Donor G-CSF	-5 Fludarabine 25 mg/m2/day IV X 1 Donor G-CSF
-4 Fludarabine 25 mg/m2/day IV X 1 Donor G-CSF	-3 Fludarabine 25 mg/m2/day IV X 1 Rabbit ATG 2.5 mg/Kg/d IV over 12 H Donor G-CSF	-2 Fludarabine 25 mg/m2/day IV X 1 Rabbit ATG 2.5 mg/Kg/d IV over 12 H Donor G-CSF Leukapheresis 1	-1 Donor G-CSF Leukapheresis 2	0 T-cell depleted Stem cell transplant	+ 1	+2
+3	+4	+5	+6	+7 Start G-CSF		

**** busulfan dosing and administration:***
Patients will have busulfan levels drawn after the first dose on day 1, with adjustments in dosing based on the pharmacokinetics of the first dose.. Dose should be adjusted if patient is >125% ideal body weight and should be calculated on adjusted ideal body weight per MSKCC"s standard of care and BMT busulfan guidelines.

***** melphalan dosing and administration:***
Dose should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight per MSKCC standard of care guidelines.

****** fludarabine dosing and administration:***

Dose should be reduced to 80% (20mg/m^2 per day) of dose for measured creatinine clearance of 40-70ml/min.

Anti-seizure prophylaxis with Keppra will be administered to all patients starting day –10 or 24 hours prior to starting busulfan for the prevention of busulfan-associated seizures.

9.2. *Peri-transplant treatment to promote engraftment*

a. Rabbit anti-thymocyte globulin* (Thymoglobulin®) and methylprednisolone (MPD) will be administered in the pre-transplant period for all patients. Patients will receive rabbit ATG (Thymoglobulin®) at 2.5 mg/Kg/day x 2 days on days -3 and -2. Methylprednisolone will be given at 2 mg/Kg/day x 2 days with the ATG administration and will be discontinued thereafter. ATG dose should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight and will be administered as per MSKCC standard of care guidelines.

Side-effects of ATG may occur (see section 11.0 "Toxicities/Side Effects"). If a reaction consisting of fever and chills occurs, then the infusion will be slowed down, meperidine could be given for the relief of the chills, and when the reaction improves the infusion rate could be slowly escalated again. Should a severe significant systemic reaction to the rabbit ATG occur, then the alternative equine ATG will be administered at doses of 15 mg/Kg/day x 2 days.

b. All patients will be treated with G-CSF (filgrastim) at 300 μg SC QD for patients ≤60kg or 480 μg SC QD for patients >60Kg. G-CSF administration will begin on day +7 and will continue until the ANC is ≥2000.

9.3. *Prophylaxis against acute graft-versus-host disease*

No further GvHD prophylaxis will be administered.

9.4. *Stem Cell Transplantation*

9.4.1. ***PBSCT***

Donor peripheral blood progenitor cells: stimulation, harvesting, isolation and T-cell depletion.

For related donors, beginning 5-6 days before the day of transplant, the normal donor will receive 10 mcg/kg of GCSF, administered subcutaneously daily for at least 5 days. On the fifth and sixth days of this course of G-CSF, the donor will undergo daily leukapheresis designed to provide a minimum of 10^9 mononuclear cells/kg of the transplant recipient's weight. For unrelated donors, the G-CSF will be administered and the leukapheresis obtained according to the National Marrow Donor Program protocol and IND.

Isolation of CD34+ hematopoietic progenitor cells with the CliniMACS TM System, Miltenyi Biotec.
The apheresis product is collected from a blood of related or unrelated donors. Aliquots of the apheresis product are collected and then tested and screened as per blood banking guidelines. The apheresis product is then prepped for the CliniMACS Cell Selection System. The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting, which can select or remove specific cell types depending on the cell-specific immunomagnetic

label used as further detailed below. The apheresis product is first co-incubated with the CliniMACS CD34 reagent (antibody-coated paramagnetic particles). Prior to and during incubation of the antiCD34 beads with the mobilized PBSC, intravenous gammaglobulin is added to the incubation fluid at a concentration of 1.5 mg IVIG/ml. After magnetic labeling and washing, the cells are passed through a high-gradient magnetic separation column in the CliniMACS clinical cell selection device. Magnetically labeled CD34+ cells are retained in the magnetized column, and $CD34^{-negative}$ cells flow through as the effluent fraction and will be discarded. The $CD34^{+positive}$ cells retained in the column are eluted by removing the magnetic field from the column, then washing the cells through the column and collecting them. The final CD34+ cell enriched product is concentrated by centrifugation and tested before final release for administration as per SOPs from the MSKCC Cytotherapy Lab Manual.

Before infusion, the CD34+ cells will be washed in normal saline for intravenous infusion containing 1% human serum albumin, and suspended in a volume of 25-50 ml for intravenous administration. Aliquots of the product are taken for in-process and final product testing will be performed as per SOPs from the Cytotherapy Lab Manual.

This study (protocol) will be conducted under BB-IND #14060. BB-IND #14060 is an existing IND that was approved by the FDA and we are currently using the Miltenyi device in the same way.

9.4.2. BMT (if PBCST not possible)

Twenty\ four to forty-eight hours after the patient has completed treatment with fludarabine, bone marrow will be harvested from the donor in the operating room according to standard procedure. The procedure for T cell depletion with soybean agglutinin (SBA) and sheep red blood cells (E) has been previously described[36, 40] as per the SOP from the MSKCC Cytotherapy Laboratory.

The amount of marrow harvested will be such to provide a minimum of 1×10^7 SBA-E- mononuclear cells/kg of the transplant recipient's weight. In very rare instances, the amount of marrow cells harvested from the donor may be inadequate to provide a high enough cell yield after the complete T-cell depletion procedure outlined above, to ensure engraftment. This can be predicted at any of the initial steps before the final RBC rosetting. In such a situation, the physician in charge of the study and/or the attending physician of the BMT service, in consultation with the laboratory investigators performing the T-cell depletion, may elect to administer either an unmodified or a partially T-cell depleted (SBA-) marrow transplant. The patient would then receive drug prophylaxis against GvHD. These patients will be statistically evaluated as recipients of T-cell depleted marrow, but will be analyzed separately in evaluation of the efficacy of the SBA-E- marrow grafts.

Transplantation of the T-cell depleted stem cells.

The CD34+T-cell depleted peripheral blood progenitor cells or the SBA-E- fraction of the bone marrow, suspended in a volume of approximately 20-50 ml will be infused intravenously over 15 minutes with monitoring of vital signs. The patient is premedicated as for blood product transfusions.

9.5. *Supportive Care*

a. *Prophylaxis against infections*

Standard of care guidelines will be followed for prophylaxis against post transplant infections by opportunistic organisms, including Pneumocystis jiroveci, fungal organisms, DNA herpesviruses and more specifically CMV.

b. *Prophylaxis against menorrhagia*

All post-pubertal females will receive prophylaxis against menorrhagia according to our standard of care guidelines.

c. *Transfusions*

All blood product transfusions will be performed as per standard of care and BMT guidelines.

d. *Nutritional support*

Nutritional status will be carefully monitored by the physician, and high-calorie parenteral alimentation will be introduced as needed. Vitamin supplements will be as clinically indicated.

Administration of Donor Lymphocyte Infusions

Calculated doses of donor lymphocytes suspended in a volume of approximately 10-30 ml will be infused intravenously over 5-10 minutes with monitoring of vital signs. The patient is premedicated as for blood product transfusions.

10.1 EVALUATION DURING TREATMENT/INTERVENTION

All patients will be closely monitored and evaluated as per MSKCC BMT standard of care guidelines. Study specific assessment schedule listed in table below.

Schedule of Study Assessments

Procedures	Pre-treat	Days Post-Trans plant														
		7	14	21	28	35	42	56	70	84	100	6 months	9 months	12 months	18 months	24 months
Window	45	(+/-) 2	(+/-) 2	(+/-) 2	(+/-) 2	(+/-) 2	(+/-) 2	(+/-) 7	(+/-) 7	(+/-) 7	(+/-) 7	(+/-) 14	(+/-) 14	(+/-) 30	(+/-) 30	(+/-) 30
Eligibility	X															
Informed consent	X															
History/Physical	X															
Dental Evaluation	X															
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CMP	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation	X															
Blood Type and screen	X															
Serology Testing (1)	X															
Pregnancy test if applicable (childbearing age)	X															
HTLV-1/2, HIV-1/2	X															
Urinalysis	X															
EKG, ECG or MUGA as needed	X															
Skeletal survey (5)	X											X		X		
PFT	X															
Chest x-ray	X															
Bonemarrow aspirate and biopsy (2)	X				X						X	X		X		X
Chimerism (Blood and BM) (3)	X				X						X	X	X	X	X	X
Myeloma Analyses (4)	X				X						X	X	X	X	X	X
GvHD evaluation (6)		X	X	X	X	X	X	X	X	X	X	X		X		
Toxicity assessment		X	X	X	X	X	X	X	X	X	X					

(1) Infectious disease markers will be performed as per each department"s guidelines or at the discretion of the treating attending.
(2) Biopsy will be stained with CD138, CD20 and kappa/lambda. Aspirate will be sent for the following tests: a. Routine staining and cell counts, b. One heparinized syringe with 5ml of marrow for flow cytometry for routine myeloma markers, c. Two syringes with EDTA and 2ml of marrow in each syringe for karyotype and FISH for chromosomal abnormalities, d. An additional 3-6ml marrow in EDTA will be drawn for correlative studies
(3) Chimerism at baseline (pretreatment) can be either blood or bone marrow. Additionally, baseline chimerism may be done at any time pre-transplant. For the day+28 time point, only bone marrow chimerism is required. Chimerism will be done at 9 months only if clinically indicated
(4) Myeloma analyses includes: serum protein electrophoresis (SPEP), serum immunofixation (IF), quantitative immunoglobulins, serum free light chain assay, β-2 microglobulin, LDH; 24 hour urine collection for total protein, creatinine clearance, urine protein electrophoresis (not required if total protein is < 10.0 mg/dl),, urine immunofixation. Myeloma analyses at 9 months only if clinically indicated
(5) Patients with positive skeletal surveys at baseline will have repeat skeletal surveys performed at 6 months and 12 months post transplant
(6) GvHD evaluations will be performed as per the ABMT service guidelines. GvHD evaluations may be conducted by phone for remote patients. This will be appropriately documented in EMR.

11.0 TOXICITIES/SIDE EFFECTS

Patients recruited to this transplantation trial are individuals who are either referred by physicians or self-referred for marrow transplantation as a potentially curative treatment for their malignancy. Prior to consideration for transplant, all patients undergo a series of 1-3 hour consultations discussing the risks and potential benefits of an allogeneic stem cell transplantation and the different procedures which will be a normal part of the transplant course. The risks and potential benefits of the transplant procedure, as well as the participation in any given research, experimental, or therapeutic protocol are also discussed.

11.1. Risks to Related Peripheral Blood Stem Cell Donors

The risks of short-term treatment with G-CSF are likely negligible. However, administration of G-CSF is frequently associated with low grade fever and low back pain which usually resolves within one day following cessation of G-CSF treatment. Furthermore, there has now been one recorded patient who developed acute splenomegaly and splenic rupture in response to high dose G-CSF. The bone pain may require treatment with analgesics. The risks of a leukapheresis are negligible, involving an occasional vasovagal response to venipuncture and the minimal hemodynamic alterations associated with single unit phlebotomies. To protect against these risks, leukophoreses are conducted in the Blood Bank Donor Room with full medical and nursing supervision and support systems to address adverse events.

For donors undergoing bone marrow harvest, the risks for the donor will mainly be those risks associated with general anesthesia. The side effects of the harvest itself will include pain at the site of harvest, i.e. posterior and/or anterior superior iliac crests, as well as minimal risks of bleeding at the site. There have been no problems with localized infections at the sites in the extensive experience of our service with marrow harvests over the last 20 years.

11.2. General Description of Risks to Recipients

Infections and hemorrhage constitute major and continuing risks throughout the period of marrow aplasia. These are, however, also the major risks associated with the primary disease. Certain opportunistic infections remain a risk in transplant patients beyond recovery of circulating leukocytes, for at least 9-12 months post-transplant, e.g. Pneumocystis carinii, cytomegalovirus and Epstein Barr virus.

Likely:

Busulfan: Myelosuppression, fatigue, not sleeping well, anorexia, nausea, vomiting, diarrhea, mucositis weight gain and swelling, changes in blood sodium level, alopecia, needing transfusions of platelets and red blood cells, fever, needing antibiotics to treat infection.

Melphalan: Myelosuppression, fatigue, not sleeping well, anorexia, nausea, vomiting, diarrhea, mucositis weight gain and swelling, changes in blood sodium level, alopecia, needing transfusions of platelets and red blood cells, fever, needing antibiotics to treat infection, transient liver dysfunction.

Fludarabine: Myelosuppression, tiredness, not sleeping well, anorexia, nausea, vomiting, diarrhea, mucositis weight gain and swelling, changes in blood sodium level, alopecia, needing transfusions of platelets and red blood cells, fever, needing antibiotics to treat infection, jaundice and elevations of liver enzymes.

Reproductive risks: Sterility. Male patients may be offered sperm banking before admission for the transplant. Possibilities of preserving the ability to have children for female patients can be discussed with the doctor. Patients should not become pregnant or father a baby while on this study because the drugs in this study can affect an unborn baby. Women should not breast feed a baby while on this study. A pregnancy test is required of all females of childbearing age before starting the transplant.

ATG: is a rabbit protein that may induce an immune response in humans. Fever, chills, changes in blood pressure, rash. Pre-medications (Benadryl, Tylenol and steroids before ATG) have been shown to be effective therapy. Side effects are usually only severe after the first dose.

Growth factor (G-CSF): bone pain, headache, body ache, feeling tired, swelling of hands/feet, nausea. These are generally mild and will go away when the growth factor is stopped.

Less Likely:

Busulfan, melphalan, fludarabine: Late effects of these three chemotherapy agents include: cataracts and under-activity of the thyroid gland. Both of these side effects can be easily treated.

Busulfan: Seizures that are generally preventable by phenytoin therapy started 24 hours prior to administration and continued for 24 hours post busulfan. Abnormal liver function, pulmonary fibrosis

Melphalan: Renal or bladder dysfunction (increased BUN, creatinine, necrosis) may be seen.

Fludarabine: confusion, numbness, loss of vision, loss of balance, difficulty walking

ATG: Prior exposure to rabbit proteins may predispose subjects to serious allergic reactions such as a drop in blood pressure, hives, bronchospasm, or serum sickness. Such reactions will be treated with epinephrine and anti-histamines. Serum sickness is an immune disease usually appearing 3-10 days after injection of a foreign serum or serum protein, with reactions such as hives, fever, swollen lymph nodes, edema, arthritis, protein in the urine, or severe inflammation of the kidney. In the event of a severe systemic allergic reaction, a trial of an alternative horse ATG will be administered. If a similar reaction occurs with the equine ATG, no further ATG will be administered.

Rare but serious:

Busulfan: high doses of Busulfan can cause Veno-Occlusive Disease (VOD). Symptoms include jaundice, liver enlargement with pain, fluid retention and increase risk of bleeding. This complication is managed by aggressive supportive care with monitoring of fluids and administration of diuretics and infusions of plasma and albumin. Veno-Occlusive Disease in its severe form can be life threatening. There is also a medication, Defibrotide, which has shown to be helpful in treating VOD.

Melphalan: pulmonary fibrosis, respiratory distress has been rarely reported. Serious hypersensitivity reactions: Edema, rash, anaphylaxis

ATG: renal toxicity, managed by Prednisone. Drop in white blood cells; drop in platelet count will be managed by transfusion therapy.

Transplant related risks:

Blood transfusions: Transfusions may induce allergic reactions. Small, subclinical pulmonary emboli may occur, but these rarely if ever require any intervention. Standard pre-medications for blood products may be used before administration of the marrow graft. Fluid overload can be managed with diuretics. Allergic reactions of variable severity can be prevented or mitigated by premedication with antipyretics, antihistamines, and narcotics. These products may also serve as vectors of serious infection (e.g., CMV, hepatitis, AIDS). To circumvent this, prospective blood and marrow donors will be screened per AABB and FAHCT guidelines. CMV antibody (-) blood products will be used in CMV(-) individuals, whenever possible, regardless of the antibody status of the marrow donor. All blood products are irradiated (3000r, ^{137}Cs) to circumvent the risk of GvHD caused by contaminating lymphocytes in the transfused fractions.

Receiving peripheral blood stem cells: The volume of the T-cell depleted peripheral blood stem cells infused is approximately 30-50 cc. Possible side effects include: changes in blood pressure,

fever, headache, shortness of breath, chills, sweats, nausea/vomiting, bad taste in the mouth. Pre-medications are given to reduce these side effects.

Receiving donor lymphocyte infusions: The volume of the donor lymphocyte infusions infused is approximately 10-30 cc. Possible side effects include: changes in blood pressure, fever, headache, shortness of breath, chills, sweats, nausea/vomiting, bad taste in the mouth. Pre-medications are given to reduce these side effects.

Graft-versus-host-disease (known as GvHD): This condition happens when the transplanted donor cells recognizes the patient"s body as foreign and attacks it. At least 1-2 out of 10 patients receiving a T cell depleted transplant will get mild to moderate GvHD. GvHD can be treated with medications (either IV or tablets). A biopsy may be necessary to make the diagnosis of GvHD.

Acute GvHD usually occurs in the first 3 months and may cause: skin rashes, nausea, vomiting, diarrhea, hepatitis, increased risk of infection, ulceration of the surfaces of the oral cavity, esophagus, and intestines, and suppressed or delayed recovery of the hematopoietic and immune system. In patients transplanted and engrafted with $SBA^{-}E^{-}$ T-cell depleted marrow from HLA 1-3 allele disparate related donors, this complication has been observed in fewer than 20% of patients and has rarely been severe. It may be fatal in at least 20-50% of cases and may also predispose to lethal infections which contribute to an additional mortality of 10-25%.

Chronic GvHD can occur any time after the first 3 months. Approximately 50% of patients with acute GvHD may also develop chronic GvHD, manifested to varying degrees by scleroderma-like changes of the skin, cirrhosis of the liver, sclerosis of lacrimal and salivary ducts, chronic inflammation and scarring of the gastrointestinal tract with consequent malabsorption and diarrhea, chronic bronchitis, and suppression of the immune system. This can be treated with standard or protocol-based experimental immunosuppression, but may be refractory.

Severe graft-versus-host disease: Rarely, GvHD can be severe or deadly. Severe acute GvHD could involve a severe skin rash like a burn, severe vomiting and/or diarrhea, liver failure and infections or bleeding. Severe acute GvHD will be treated with intense immunosuppressive therapy according to standard clinical practice or other experimental protocol. Severe chronic GvHD could involve similar symptoms but may produce other symptoms such as severe skin changes, severe dry eyes and weight loss.

Steroids, as treatment for GvHD: inability to sleep, high blood sugar, puffiness of the face, changes in the skin, high blood pressure, increased risk of infection, weight gain, reduced growth in children, thinning of the bones

Infections or bleeding: Full recovery of blood counts may take months. Full recovery of the immune system may take months to a few years. For this reason patients will be at increased risk of infections and bleeding. Medications are given to reduce the chance of those infections. Patients will receive treatment if they do get an infection and most infections can be treated successfully with antibiotics. Patients will stay in the hospital longer or be readmitted if found to have an infection. Patients are watched closely for bleeding and given platelet transfusions to prevent serious bleeding, but minor bleeding may occur.

Serious infections or bleeding: Some infections are very difficult to treat, even with strong antibiotics. Rarely, serious infections can be passed on by the transfusion of blood products. Serious bleeding can happen in spite of platelet transfusions. Rarely infections or bleeding are lethal.

Potential sensitization to murine proteins: Mouse protein (the anti CD34 antibody used in this device is of murine derivation) is used in the CliniMACS processing procedure. Marrow cells are also separated on bovine serum albumin gradients and exposed to sheep red cells to remove rosetting populations. It is possible that patients may have pre-existing immunity to these proteins and may be at risk for allergic reactions during infusion of the processed marrow and/or peripheral blood. No allergic reactions have been noted with infusions of cells processed by the CliniMACS system in clinical studies or from infusion of cells recovered by depletion of SRBC-rosette-positive cells. Precautions for an allergic event will be taken during the infusion of the processed cells.

Pneumocystis jiroveci prophylaxis: The risk of trimethoprim and sulfamethoxazole in the doses given are primarily hypersensitivity reactions and signs of folate deficiency. Any patient with known hypersensitivity to these compounds will not receive these drugs. The risks of parenteral pentamidine are primarily hypotension and hypoglycemia both of which will be monitored during and following administration of the drug. Hypokalemia or hypomagnesemia associated with prolonged QT syndrome or Torsade de pointe necessitates strict electrolyte monitoring. The risks of aerosolized pentamidine are mild bronchospasm primarily observed in (prior) tobacco abusers and easily managed with bronchodilator therapy.

Risk of a secondary cancer different from multiple myeloma may happen after chemotherapy. The risk of developing a secondary cancer of the skin, cervix, etc., which has been seen in other studies of similar transplants, is less than 5%. There is a special concern in patients who receive a T cell depleted transplants because there is a risk of having a cancer of the lymph nodes (lymphoma) caused by the Epstein Barr virus (EBV). This virus causes mononucleosis in healthy people. Treatment of EBV lymphoma includes Donor Leukocyte Infusion (DLI) and Rituximab.

Graft Failure: Bone marrow graft may fail to grow. Past experience suggests that this may occur in about 10% of patients. If graft failure occurs, it is unlikely that bone marrow will recover and a second transplant with stem cells from the same donor or a different donor will be needed.

Progression of Disease and Relapse of Multiple Myeloma is a risk even if the transplant is initially successful. Patients on the relapsed cohort may be treated prophylactically with Revlimid maintenance therapy post transplant.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

DEFINITIONS OF DISEASE STATUS

Patients at each data collection period are classified into one of the following states.

1. Complete Response (CR)

CR requires ***all*** of the following:

- Absence of the original M-protein in serum and urine by routine electrophoresis and by immunofixation maintained for a minimum of six weeks. The presence of new monoclonal bands consistent with oligoclonal immune reconstitution does not exclude CR.
- Less than 5% plasma cells in a bone marrow aspirate and also on trephine bone biopsy, if biopsy is performed. Confirmation with repeat bone marrow studies is not needed if the last bone marrow assessment was negative.
- No increase in size or number of lytic bone lesions on radiological investigations, if performed (development of a compression fracture does not exclude response).

Patients in whom some, but not all, the criteria for CR are fulfilled are classified as very good partial response or partial response (see below), providing the remaining criteria satisfy the requirement for partial response. This includes patients in whom routine electrophoresis is negative but in whom immunofixation has not been performed.

2. Very Good Partial Response (VGPR)

- Reduction of serum M-protein on electrophoresis by at least 90% with a urine M-protein <100mg/24hours, but detectable immunofixation.

3. Partial Response (PR)

PR requires the following:
- Greater than or equal to 50% reduction in the level of the serum M-protein, maintained for a minimum of six weeks.

OR

- Reduction of urine M-protein to less than 200 mg/24 hours, maintained for a minimum of six weeks
- No increase in size or number of lytic bone lesions on radiological investigations, if performed (development of a compression fracture does not exclude response).

For patients with nonsecretory myeloma, free light chain (FLC) levels can be used to determine a partial response. A 50% decrease in the difference between kappa and lambda FLC levels denotes a PR. If the FLC levels were unmeasurable at baseline, a 50% reduction in bone marrow plasma cells is acceptable as long as the original bone marrow contained at least 30% plasma cells. All PR require a 50% reduction in size of any soft tissue plasmacytomas

Patients in whom some, but not all, the criteria for PR are fulfilled are classified as minimal response (see below), providing the remaining criteria satisfy the requirements for minimal response.

4. Minimal Response (MR)

Minimal response requires the following:
- 25-49% reduction in the level of the serum M-protein.

OR

- Urine M-protein which still exceeds 200 mg/24 hours
- 25-49% reduction in the size of soft tissue plasmacytomas (by radiography or clinical examination).
- No increase in the size or number of lytic bone lesions on radiological investigations, if performed (development of a compression fracture does not exclude response)

5. Stable Disease (SD)

Stable values (within 25% above or below value at time response is assessed) maintained for at least three months.

6. Relapse

Relapse from CR requires ***one or more*** of the following:

- Reappearance of serum or urine paraprotein on immunofixation or routine electrophoresis, confirmed by at least two further consecutive investigation with 2-4 weeks and excluding oligoclonal immune reconstitution
- Greater than or equal to 5% plasma cells in a bone marrow aspirate or on trephine bone biopsy
- Development of new lytic bone lesions or soft tissue plasmacytomas or definite increase in the size of residual bone lesions. Development of a compression fracture does not exclude continued response and may not indicate progression
- Development of hypercalcemia (corrected serum Ca > 11.5 mg/dL or > 2.8 mmol/L) not attributable to any other cause

7. Progressive Disease (PD)

For patients not in CR, progressive disease requires ***one or more*** of the following:

- > 25% increase in the level of the serum M-protein, which must also be an absolute increase of at least 0.5 g/dL and confirmed by at least one repeated investigation
- An absolute increase in urine M-protein of at least 200 mg/24 hours and confirmed by at least one repeated investigation
- > 25% increase in plasma cells in a bone marrow aspirate or on trephine biopsy, which must also be an absolute increase of at least 10%
- Definite increase in the size of existing bone lesions or soft tissue plasmacytomas
- Development of new bone lesions or soft tissue plasmacytomas
- Development of hypercalcemia (corrected serum Ca > 11.5 mg/dL or > 2.8 mmol/L) not attributable to any other cause
- Development of a compression fracture does not exclude continued response and may not indicated progression

Definition of events in the post-transplant course important for analysis and treatment

12.1. Regimen-related and transplant-related mortality

Regimen related toxicity (RRT) refers to those toxicities that can be attributed directly to the preparative regimen (including chemotherapeutic agents, ATG, and donor lymphocyte infusion).

Transplant-related mortality (TRM) includes the RRT and other fatal complications resulting from the allogenic transplant such as graft failure, GvHD, hemorrhages, and infections. One year post TRM is the primary endpoint of this study.

The grading for monitoring the morbidity and mortality will be based on the NCI/CTEP common toxicity criteria version 4.0. This will include assessment of severity and duration of oral mucositis and sequelae specifically parenteral opioid analgesic use, TPN use, febrile neutropenia, hospital days and intubation.

12.2. Immunologic Reconstitution

Our previously reported analyses have identified time points at which the various immunologic functions can be expected to return. Patients receiving T-cell depleted transplants from HLA-identical related donors can be monitored at fewer time points without compromising our ability to obtain critical information. However, patients receiving transplants from partially HLA-matched related donors will be evaluated more frequently, as graft rej ection, late graft failures, more pronounced immune dysfunction, and more patient to patient variability have been observed.

Immunophenotyping of T-cells, B-cells, and NK cells, will be performed at approximately 3, and every 3-6 months post-transplant until normal values are reached.

T-cell proliferations in response to PHA will be performed at approximately 3 months post-transplant and every 3-6 months thereafter until normal values are reached. These tests are not required when a patient"s absolute lymphocyte count is less than 0.5 K/mcL.

Immunoglobulin levels will be tested at 6 months, 9 months (if clinically indicated), 12 and 18 months post-transplant and thereafter as clinically indicated. Patients with normal total IgG levels and PHA response within the 25th percentile of normal may be re-immunized with diphtheria and tetanus toxoid, killed polio vaccine, hepatitis B vaccine, as well as the Haemophilus influenzae type B conjugate vaccine. Specific antibody production to tetanus and Hepatitis B is then measured.

Antigen-specific T-cell immune reconstitution will be performed as described in detail under „correlative studies".

12.3 Engraftment and chimerism

Engraftment will be documented by analysis of T cells and bone marrow cells for chimerism by standard cytogenetic studies at 1 month, 100 days, 6 months, 9 months (if clinically indicated), and 12 months, 18 months, 24 months (if clinically indicated) post transplant or as needed thereafter. T-cell chimerism analysis is not required when a patient"s absolute lymphocyte count is less than 0.5 K/mcL.

12.4 Graft failure or rejection

Primary non-engraftment is diagnosed when the patient fails to achieve an ANC $\geq$500/μl at any time in the first 28 days post-transplant. If the patient's myeloma recurs during this interval, the patient is scored as having refractory myeloma. In such a situation, the absence of donor hematopoiesis is not evaluable for graft failure or rejection. If host T-cells capable of specifically inhibiting donor hematopoietic progenitor growth in vitro are concurrently detected during graft failure, a presumptive diagnosis of immune mediated rejection is made. If (1) after achievement of an ANC $\geq$500/mm^3, the

ANC declines to <500/mm^3 for more than 3 consecutive days in the absence of relapse, or, (2) there is absence of donor cells in the marrow and/or blood as demonstrated by chimerism assay in the absence of relapse, a diagnosis of secondary graft failure is made. If, however, recurrence of host myeloma is detected concurrently, the patient is not evaluable for graft failure or rejection.

Patients with evidence of graft failure without evidence of recurrence of host myeloma will have additional studies drawn to ascertain cause and define relevant histoincompatibilities. These analyses may include (1) Evaluation of bone marrow aspirates and biopsies for residual or recurrent myeloma, when indicated, (2) Culture and/or molecular analyses of marrow and blood for viral pathogens potentially causing graft failure including CMV, HHV6 and parvovirus B 19, (3) Immunophenotypic and genetic analysis of circulating T-cells and NK cells to ascertain their origin and potential function, (4) Analysis of the functional activity of residual circulating lymphocytes to determine whether and to what degree they exhibit cytotoxic or cyto-inhibitory activity against donor host or third party PHA-stimulated blasts or clonogenic hematopoietic progenitor cells. If donor-specific reactivity is identified, attempts will be made to identify targeted specificities (HLA or minor alloantigens) whenever possible.

Patients who suffer graft failure will be considered for a secondary transplant. The need for additional immunosuppression or treatment for viral infection prior to the secondary transplant will be determined by the results obtained from chimeric and viral studies.

12.5. Graft-versus-host disease

Standard BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al[68] will be used to establish and grade acute GvHD.

To determine the severity of acute GvHD, data will be collected approximately weekly to characterize the severity of symptoms and signs caused by GvHD and to evaluate possible confounding factors. Real time data collection will include descriptive characteristics of rash and estimated body surface area involved, extent of dermal/epidermal separation, identification of concomitant causes of increased bilirubin other than GvHD, presence or absence of nausea, vomiting or anorexia persistent after engraftment, peak diarrhea volume with annotations concerning the presence after engraftment, peak diarrhea volume with annotations concerning the presence or absence of urinary mixing and estimates of true diarrhea volume, presence or absence of abdominal cramps, presence or absence of frank stool blood or melena, concomitant causes of GI symptoms other than GvHD, biopsy results, identification of any agents used for treatment and autopsy results.

Patients will be observed for acute and/or chronic GvHD. Graft-versus-host disease occurring after DLI infusions will be analyzed separately.

Patients with moderate to severe acute GvHD (grade II-IV) will be treated in standard fashion with high-dose I.V. methylprednisolone (1-2mg/kg/day) or in combination with other immunosuppressants as per ongoing trials on GvHD. Patients failing to respond to steroids will be considered for treatment with experimental treatments available at the time of diagnosis of GvHD.

Chronic GvHD will be diagnosed and graded according to the criteria of Sullivan[69] treated with standard or experimental immunosuppressive therapy. Treatment will consist of corticosteroids,

cyclosporin A or azathioprine, or combinations of these agents. Other novel treatments could be used if available, i.e. thalidomide and psoralen/ultraviolet A phototherapy (PUVA).

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for patient/subject eligibility (e.g. a change in diagnosis), the patient will be removed from the study. Also patients may be removed from the study if requested by the patient. Management will depend on where they are in their treatment course. Such patients will receive appropriate supportive care. The PI may also remove patients from the study for noncompliance.

14.0 BIOSTATISTICS

This is a phase 2 study designed to examine the efficacy and safety of chemotherapy followed by T-cell depleted transplant and donor leukocyte infusions for patients with relapsed or high-risk multiple myeloma. The primary objective is to assess the overall and progression free survival from the time of transplant in the relapsed and high-risk groups. A maximum of 30 patients will be accrued into each group. The primary endpoint of the study is one-year progression-free survival from the time of transplant. An event is defined as a disease progression or death. The accrual period will be approximately three years with an additional 24 month follow up after the accrual has been completed.

For the relapsed multiple myeloma cohort, a single stage design that differentiates between one -year PFS rates of 0.30 and 0.54 will be used to assess treatment efficacy. At the conclusion of the study, if at least 13/30 patients were recorded progression-free at 1 year, then the treatment will be declared a success. The probability of declaring the treatment a success is ≤ 0.10 when the one-year PFS in the population is 0.30 and increases to ≥ 0.90 when the one-year progression-free survival is 0.54.

Recent data at MSKCC suggests that the addition of immune gammaglobulin (IVIG) added to the incubation fluid at a concentration of 1.5 mg IVIG/ml during incubation of the antiCD34 beads with the mobilized PBSC results in a reduction in the rate of grade 2-4 acute graft versus host disease (GVHD). A total of 17 out of the 30 patients in the relapsed cohort were treated without the IVIG addition.

Since DLI and the lack of IVIG may both increase a patient"s risk of GVHD, it is imperative to understand the risk that is attributable to the TCD+DLI treatment strategy and not the change in CD34+ preparation. Therefore this cohort will be expanded so that the outcomes can be evaluated on patients who all receive the IVIG addition. The outcomes for the N=17 cohort who did not receive IVIG will be reported separately. The stopping rules below have been extended to the 47 patients enrolled in the relapsed arm.

For the high-risk multiple myeloma cohort, a single stage design that differentiates between one-year PFS rates of 0.45 and 0.69 will be used to assess treatment efficacy. At the conclusion of the study, if at least 18/30 patients were recorded progression-free at 1 year, then the treatment will be declared a success. The probability of declaring the treatment a success is ≤ 0.10 when the one -year PFS in the population is 0.45 and increases to 0.89 when the one-year progression-free survival is 0.69.

In order to reduce patient risk, the study design includes early termination in the event of excessive graft failure, graft versus host disease, or early treatment related mortality during the accrual period. Stopping rules for excessive failure and the corresponding power calculations are applied to each patient cohort separately. The stopping rules are provided in the tables below and consider only the univariate failure probabilities.

Relapsed Multiple Myeloma

Failure type	# of failures needed to stop the study	Projected probability of failure in the population	Probability of study completion (based on projection)
Graft failure	2 in the first 26 patients	0.15	0.03
	3 in the first 47 patients	0.02	0.92
Treatment Related Mortality	3 in the first 8 patients 4 in the first 14 patients 5 in the first 20 patients 6 in the first 27 patients 7 in the first 34 patients **8 in the first 41 patients** **9 at any point**	0.30	0.02
		0.10	0.88
Acute Graft Versus Host Disease (Grade 4)	3 in the first 12 patients 4 in the first 20 patients 5 in the first 29 patients 6 in the first 38 patients **7 at any point**	0.25	0.02
		0.07	0.89
Chronic Graft Versus Host Disease (Severe)	3 in the first 8 patients 4 in the first 14 patients 5 in the first 20 patients 6 in the first 27 patients 7 in the first 34 patients **8 in the first 41 patients** **9 at any point**	0.30	0.02
		0.10	0.88

High-risk Multiple Myeloma

	# of failures needed to stop the study	Projected probability of failure in the population	Probability of study completion (based

Failure type			on projection)
Graft failure	2 in the first 26 patients	0.15	0.08
	3 in the first 30 patients	0.02	0.90
Treatment Related Mortality	3 in the first 8 patients 4 in the first 14 patients 5 in the first 21 patients 6 in the first 27 patients 7 in the first 30 patients	0.30	0.10
		0.10	0.90
Acute Graft Versus Host Disease (Grade 4)	3 in the first 12 patients 4 in the first 20 patients 5 in the first 29 patients 6 in the first 30 patients	0.25	0.09
		0.07	0.92
Chronic Graft Versus Host Disease (Severe)	3 in the first 8 patients 4 in the first 14 patients 5 in the first 21 patients 6 in the first 27 patients 7 in the first 30 patients	0.30	0.10
		0.10	0.90

At the conclusion of the study, Kaplan-Meier estimates of overall and progression-free survival will be computed over time. In addition, the probability of treatment related mortality and acute and chronic graft-versus-host disease will be calculated using the cumulative incidence function.

Correlative studies are undertaken to explore the role of four CT antigens (CT7, CT10, MAGE-A3, and NY-ESO-1) for disease progression. The antigen expression, evaluated by quantitative PCR, will be recorded at baseline and multiple time points post-baseline (section 10) and a time-dependent Cox model will be used to assess the association of each antigen with progression free survival.

Finally, to account for patients that relapse and are put back into remission through DLI, the current myeloma free survival curve will be computed. This curve estimates the probability a patient is alive in an original remission or in subsequent remission after treatment with DLI at a given time after transplant.[70]

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study.

Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.3 Randomization

This research study does not require a randomization.

16.1 DATA MANAGEMENT ISSUES

A research study assistant (RSA) will be assigned to the study. The responsibilities of the RSA will include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem and problem resolutions, and coordination of the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Full sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, and more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring Plans (DSM) at Memorial Sloan-Kettering cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: Http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://mskweb2.mskcc.org/irb/index.htm.

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center‟s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

17.1 PROTECTION OF HUMAN SUBJECTS

Consent Process: Participation in this study is voluntary. All patients will be required to sign a statement of informed consent which must conform to MSKCC IRB guidelines and explain the risks and potential benefits of the transplant procedure.

Alternatives: Enrollment in this study is voluntary. Alternative treatment options will be presented to the patient prior to taking part in this study. Alternative treatment options may include getting a transplant from a volunteer unrelated donor, if one is available; getting treatment for the cancer with either chemotherapy or a transplant without being on a study; taking part in another study; or getting no treatment.

Costs: The patient"s health plan/insurance company will need to pay for all of the costs of treatment in this study. The patient will be responsible for the costs of standard medical care, all hospitalizations and any transplant complications. Pre-authorization for the transplant will be cleared with the health plan/insurance company prior to admission. Patients will not be paid for taking part in this study. Research tests will be done at no cost to the patient.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential.

17.2 Privacy

MSKCC"s Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.3 Serious Adverse Event (SAE) Reporting

Potentially serious toxicities are an expected part of transplant therapy. Grade 4 toxicities will be recorded and reported as part of the analysis of study outcome. In addition, the reportable serious adverse events (SAEs) will be defined according to current MSKCC BMT program guidelines which is included as an appendix.

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant"s last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 „Reporting of Serious Adverse Events", the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject"s initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject"s condition

- Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI"s signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office

17.2.1

This protocol has an IND therefore, the SAE will also be reported to the FDA through the IND Office and the report will include the FDA assigned IND number and name.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

Appendix A: The Adult and Pediatric BMT Adverse Event Reporting Standard Operating Procedures.