# A Phase II Trial of Busulfan, Melphalan, and Fludarabine with Peri-transplant Palifermin, followed by a T-Cell Depleted Hematopoietic Stem Cell Transplant from HLA Matched or Mismatched Related or Unrelated Donors in Patients with Advanced Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML) Evolved from MDS

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 single arm phase II trial to assess the efficacy (decrease the transplant-related morbidity) and safety of peri-transplant Palifermin in combination with 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 advanced MDS and AML evolved from MDS. The addition of Palifermin is to decrease the toxicity and the infection rate associated with this regimen and transplant type and to foster earlier immune reconstitution.

Candidates for this trial will include patients with MDS: refractory anemia with excess blasts type 1 and 2, and AML evolved from MDS who are in hematologic remission, in a second refractory cytopenia phase (no excess of blasts) or have a persistent blast count of 5-9% in the bone marrow after high or low dose chemotherapy. Pre-transplant chemotherapy prior to cytoreduction is required to participate in this trial. Patients with refractory disease and ≥10% blast in the peripheral blood or bone marrow are not eligible for this trial.

Hematopoietic stem cell donors for this trial will include individuals who are 10/10 HLA matched or up two-antigen mismatched at the HLA A, B, C, DRB1, and DQB1 loci, as defined by high resolution methods.

All patients will receive Palifermin (60 mcg/kg/day) for 3 days before the start of chemotherapy and for 3 days starting the day of transplantation. All patients will be conditioned for transplantation with busulfan (Busulfex®) (0.8-1.0 mg/Kg/dose Q6H x 12 doses), melphalan (70 mg/m$^2$/day x 2 doses) and fludarabine (25mg/m$^2$/day x 5 doses). Doses of busulfan will be adjusted according to plasma levels. All patients will also receive ATG (Thymoglobulin®) prior to transplant to promote engraftment.

The preferred source of stem cells will be peripheral blood stem cells (PBSC) induced and mobilized by treatment of the donor with G-CSF. 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 soybean agglutinin and E-rosetting (SBA-E-). No drug prophylaxis against GvHD will be administered post transplant. Patients will also receive G-CSF post-transplant to foster engraftment.

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, incidence of bacterial, fungal, viral and parasitic infections, transplant related and relapsed related mortality, and overall and disease-free survival.

The data analysis and data monitoring will conducted separately in each HLA group. A maximum of 31 patients in the HLA matched donor group and 21 patients in the HLA mismatched donor group are planned for accrual onto the study. It is anticipated that accrual will last three years and each patient will be followed for a minimum of two years if he/she has not relapsed. The primary endpoint of the study is one-year treatment related mortality (TRM).

For patients in the HLA matched donor group, a study design that differentiates between treatment related mortality probabilities of 0.10 and 0.30 will be used to assess treatment

efficacy. At the conclusion of the study, if at least 25/31 patients do not experience treatment related mortality within the first year of transplant, the treatment will be declared a success in controlling treatment related mortality. The probability of declaring the treatment a success for TRM is 0.09 when the one-year TRM in the population is 0.30 and increases to 0.90 when the one-year TRM in the population is 0.10.

## 2.1 OBJECTIVES AND SCIENTIFIC AIMS

This study is a prospective phase II trial of the safety and efficacy of the addition of peri-transplant Palifermin to a chemotherapy-based cytoreduction using busulfan, melphalan, fludarabine, and ATG with T cell depleted hematopoietic stem cell transplants (HSCT) from histocompatible related and unrelated donors, in patients with advanced myelodysplastic syndromes. This study follows our previous phase II trial with the same preparative regimen and the same type of transplant but without Palifermin in a specific disease group.

Primary objective of this trial is:

1. To reduce the early transplant-related mortality.

Secondary objectives of this study are:

1. To improve the quality of immune reconstitution following transplantation
2. To reduce the incidence rate of fatal post transplant infectious complications.
3. To improve the rates of overall (OS) and disease-free survival (DFS).
4. To assess the incidence of and severity of GvHD.
5. To assess the incidence and severity of mucositis

## 3.0 BACKGROUND AND RATIONALE

### *3.1. Advanced Myelodysplastic Syndromes*

Myelodysplastic syndromes (MDS) are hematopoietic stem cell disorders characterized by cytopenias with a high risk of transformation to acute leukemia. The median survival for MDS patients with refractory cytopenias without an excess of blasts is about 5 years and some of the variants such as the 5q- syndrome are very responsive to non-transplant options such as lenalidomide and can survive for many years [1]. The more advanced forms of MDS, as defined by an increase in the blast count, have a shorter survival. The median survival for patients with refractory anemia with excess of blasts (RAEB-1) is 1.5 years and for patients with RAEB-2 is one year [2]. The most common complication in patients with RAEB-1 and RAEB-2 is transformation to acute myelogenous leukemia (AML). Although a variety of non-transplant options are currently being developed and survival has somehow improved, none of these treatments is curative in these patients with advanced MDS and AML evolved from MDS. Treatment of these patients with induction chemotherapy and then with high dose chemotherapy followed by autologous stem cell rescue has resulted in DFS at 5 years of less than 20% [3, 4].

### *3.2. Allogeneic Hematopoietic Stem cell Transplantation for MDS*

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment available for patients with myelodysplastic syndromes[5-15]. However, the success rate in these patients has been hindered by two major problems: a high transplant-related mortality and a high post transplant disease relapse, particularly in patients with advanced MDS. The early allogeneic HSCT studies focused in transplantation of bone marrow from HLA-matched sibling donors following myeloablative conditioning regimens utilizing either total body irradiation and

cyclophosphamide or busulfan and cyclophosphamide. The best long term disease-free survival rates after HLA-matched unmodified marrow grafts were seen in younger patients (<40 years of age). However, one of the main obstacles limiting the success of allogeneic marrow transplantation was the high incidence of transplant-related mortality, due mostly to graft-versus-host disease (GvHD) and associated complications [5-7].

In order 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 (> 40 years of age), who constitute the majority of patients with MDS, 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. To this end, several groups have developed and evaluated combinations of immunosuppressive drugs for the prevention of GvHD [16-26]. 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 [27-34]. For example, 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% [24]. 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% [35].

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 [36, 37]. 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 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% [35, 38, 39]. These rates of graft failure are comparable to those observed following unmodified transplants from such donors [40]. Additionally, Aversa et al [41-43] (54-56) demonstrated that when G-CSF mobilized peripheral blood stem cells are utilized as the source of hematopoietic stem cells for a 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 [41]. Based upon this observation, G-CSF mobilized peripheral blood stem cells are currently the preferred source of hematopoietic cells for T cell depleted transplants.

Another approach to overcome the problem of GvHD and the high incidence of transplant-related mortality in allogeneic SCT is the use of non-myeloablative or reduced intensity conditioning regimens. The administration of lower doses of the agents used in the conditioning regimen to decrease the direct organ toxicity, infections, and acute GvHD, particularly in older patients, has resulted in an improvement of regimen related toxicities and acute GvHD, but not in

significant improvement in the incidence of chronic GvHD [44-49]. Moreover, the incidence of post transplant relapse, particularly in patients with advanced MDS, is higher in non-myeloablative transplant as compared to myeloablative transplants. A recent retrospective comparative analysis of the European experience of myeloablative versus non-myeloablative allogeneic SCT from HLA matched siblings in 836 patients with MDS revealed that the incidence of relapse after non myeloablative conditioning was higher whether the patient is in remission or has persistent disease after induction chemotherapy [47]. A major limitation of non-myeloablative transplants is that only patients with an HLA matched related or unrelated donor can benefit form this type of preparative regimen, as the risk of GvHD is too high in HLA mismatched transplants [47]. In contrast, T cell depleted transplants do not have the limitation of HLA matching.

The other major problem limiting the success of allogeneic SCT in MDS, particularly in patients with advanced forms of MDS (≥5% blasts in the bone marrow or >1% blasts in the peripheral blood), and AML transformation is the high rate of post transplant relapse ranging from 30 to 50% [4-15]. This is in contrast to the lower relapse rate and better disease free survival in patients with refractory anemia, refractory cytopenias with multilineage dysplasia with or without ringed sideroblasts [50]. Different approaches have been tried to reduce the incidence of post-transplant relapse in advanced MDS. The use of more intensive preparative regimens combining total body irradiation and busulfan reduced the incidence of relapse but resulted in an increased regimen related morbidity and mortality [51]. The use of induction chemotherapy to achieve remission before the administration of cytoreductive therapies preceding the allogeneic transplant remains controversial. Several retrospective studies of myeloablative non T cell depleted transplants in patients with advanced MDS and AML evolved from MDS have shown discordant results. Data from the European and the French Registry showed a better DFS following allogeneic BMT in MDS patients who were transplanted in hematologic remission as compared to patients transplanted with refractory disease (DFS at 3 years of 25% versus 15%) [4, 53]. However, induction chemotherapy was associated with a morbidity and mortality of 5 to 15%. More recently the Fred Hutchinson Cancer Center reported their experience in 125 patients with advanced MDS and AML evolved from MDS who received transplants from HLA identical siblings or unrelated donors after preparation with myeloablative conditioning regimes [52]. Thirty–three of them received pre-cytoreduction induction chemotherapy. The relapse-free survival at 3 years was similar in patients receiving and not receiving induction chemotherapy, 13% and 26% respectively.

Since the focus of this institution's transplant program has been T cell depletion, patients with MDS have been transplanted with this type of allograft. Our initial experience with T cell depleted transplants in patients with advanced MDS showed a high incidence of relapse and graft failure. Subsequent patients were treated with chemotherapy before undergoing a T cell depleted SCT. Our results summarized below showed that patients with advanced MDS have better transplant outcomes if they are transplanted in remission after chemotherapy. Also, in our earlier trials the preparative regimen was TBI based and throughout the years this regimen was modified to reduce some of the complications associated with T cell depleted transplants, such as graft rejection, as described above. Because MDS patients are mostly older patients (>age 40) and some of them are not eligible to receive total body irradiation, our institution also 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 overall experience of T cell depleted SCT in patients with advanced MDS is described in the subsequent paragraphs and the results in patients treated with the chemotherapy only preparative regimen are summarized in the next section.

From 1985 to 2004, 77 patients with advanced MDS underwent TCD allogeneic SCT at this institution, 49 from HLA matched siblings and 28 from unrelated donors [54, 55]. All patients received a myeloablative conditioning and the majority received a total body irradiation regimen combined with chemotherapy. Fewer patients received conditioning with a busulfan-containing regimen; 3 out 49 in the HLA matched sibling transplants and 11 out 17 in the unrelated transplants. The age range was 13 to 61 with a median of 48 in the HLA matched sibling group and an age range of 4 to 61 with a median of 61 in the unrelated group. Fifteen patients did not receive any chemotherapy prior to conditioning and the majority of the remaining 61 patients received induction dose of chemotherapy and a few received low dose chemotherapy. Of the patient who received chemotherapy before cytoreduction for transplantation, 46 were in remission (40) or in a second refractory anemia phase (6); and 16 had failed to respond to chemotherapy or their disease progressed before conditioning. The 5 year DFS for patients in remission or in a second refractory cytopenia phase was 50%, 23% for patients with residual disease, and 0% for patients with refractory disease. The relapse rate after T cell depleted myeloablative transplants in our MDS patients in remission or second refractory cytopenia phase is lower or at least similar to that seen in recipients of T cell replete myeloablative transplants reported by other centers. The incidence of acute and chronic GvHD in this series of 77 patients was low, 11 patients developed acute GvHD (grade 1 to 3), and only 3 chronic GvHD (two patients after the infusion of donor lymphocytes). GvHD was the cause of death in two patients.
The most common causes of non-relapse mortality in this series were graft failure and infections. Graft failure was a significant complication in the untreated group despite rejection prophylaxis with ATG. Most of these patients had active disease and had a high blast count before transplantation. The most likely mechanism was persistent disease. Graft failure attributed to persistent disease has been also noted after T cell replete myeloablative and non-myeloablative transplants for advance MDS[14.] Bacterial, fungal and viral infections in the post transplant period were the most common cause of non-relapse deaths. Infectious complications occurred in the early (<3 months) and particularly in the late post transplant period (>3 months). All patients who died of infections in the late post transplant period had poor immune function as determined by low CD4 counts and poor response to mitogens. Forty eight percent of patients were ≥50 years old. Studies at our institution have shown that older patients have a poor immune recovery posttransplant[56], suggesting that recipient factors such as thymic function are important contributors to the recovery of the immune function following allogeneic SCT.

In summary, this study in a relatively large series with a long follow-up over 19 years showed that patients with advanced MDS can have a significant long-term disease-free survival after a T cell-depleted myeloablative allogeneic SCT if they receive chemotherapy and achieve sustained remission or a second refractory cytopenia phase before undergoing cytoreduction for transplantation. Patients with limited disease also had reasonable DFS, whereas patients with untreated or refractory disease had a poor outcome. The survival benefit in the patients transplanted in remission was largely due a significant decrease in GvHD and post-transplant relapse. Opportunistic infections were an important cause of non-relapse mortality in older patients.

*3.3. Summary of results of earlier phase II trial of chemotherapy only cytoreductive regimen comprised of busulfan, melphalan and fludarabine followed by T-cell depleted transplants from matched related, mismatched related, matched unrelated or mismatched unrelated donors.*

This preparative regimen was designed as an alternative to TBI containing regimen that could be offered to patients who were not eligible to receive TBI because of previous exposure to high

dose radiation therapy or because of older age. The total dose of busulfan was decreased to 10 doses of 0.8-1 mg/kg every 6 hours, versus the standard myeloablative dose of 16 doses of 0.8-1 mg/kg every 6 hours, to reduce the toxicity of non-hematopoietic tissues, but maintaining its myeloablative and lymphoablative potency in combination with melphalan, fludarabine and ATG to allow the engraftment of a T cell depleted hematopoietic SCT.

Overall Results

The previous study (without Palifermin), in which this proposed trial is based, included 59 patients; 6 children and 53 adults. The median age was 54.2 years with a range of 0.6 to 71.3; thirty-six were 50 or older. The diagnoses included: MDS and AML evolved from MDS (41 patients), de novo AML in CR1 or 2 (10 patients), ALL (4 patients), CML (1 patient), NHL (2 patients), and MPD (one patient). The donors were HLA matched (A, B, C, DR, DQ by high resolution) related (16), mismatched related (5), matched unrelated (20), and mismatched unrelated (18 patients). Except in two patients, the source of stem cells was peripheral blood. Fifty seven patients are evaluable for outcomes and have 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 3 patients achieved durable engraftment. Three recipients of unrelated HLA disparate transplants experienced primary (one patient) or late (2 patients) graft failure, of whom 2 achieved durable engraftment after a secondary transplant. Acute GVHD II or III developed in 3 patients and chronic GVHD in 2 patients (1 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 initial objectives of securing consistent engraftment with a GF <10% and grade II-IV acute GVHD in 1 HLA disparate locus recipient have been met. As far as efficacy, the DFS was disease and stage dependent. The most significant results which are described below were in the group of patients with MDS and AML evolved from MDS. This preparative regimen was not effective in preventing relapse in patients with advanced ALL and NHL, as their disease relapsed in all 4 patients.

**Results in MDS patients**

With the exception of one patient with RAEB-2 who had 10% blasts, MDS patients whose disease status following chemotherapy and prior to conditioning was RAEB-2 or AML were not enrolled in this trial based upon our earlier observations showing that the relapse rate was unacceptably high in these patients. MDS/AML patients enrolled on this protocol included 41 patients with 19 males and 22 females, aged 0.6 – 71 years of age (median 56 years). This included 3 patients < 20 years, 9 patients aged 20-49 years, 20 patients aged 50-59 years and 9 patients aged > 60 years, with 29 patients (70%) 50 years or older. Twenty three patients had primary MDS and 18 therapy-related. The MDS subtype at diagnosis included: refractory cytopenia (15 patients), RAEB-1 (9 patients), RAEB-2 (9 patients), 4 patients in AML, and 4 patients with JMML/CMMoL (4 patients). Of these 41 patients, 14 patients had normal cytogenetics and 27 had abnormal cytogenetics with 14 patients with chromosome 7 abnormalities, 7 patients with 11q abnormalities, 6 patients with chromosome 5 abnormalities and 2 patients with trisomy 8. Thirty-six patients (84%) received treatment with chemotherapy prior to proceeding to conditioning. Disease status pre-transplant included: 35 patients with minimal disease - CR (14 patients), RA (21 patients) – and 6 patients with persistent but limited disease – RAEB-1 (5 patients) and RAEB-2 (1 patient).

Donors for these patients included 11 HLA-matched related donors, 15 HLA-matched unrelated donors, 2 HLA-mismatched related donors, and 13 HLA-mismatched unrelated donors. Overall 26 patients (63%) had HLA-matched donors and 15 patients (37%) had HLA-mismatched donors. HLA-mismatch included class I for 10 patients, class II for 3 patients, and class I and II for 2 patients. The matching level as per the 5 pairs of class I and II HLA antigens was; 9/10 antigen matched in 8 patients and 8/10 antigen matching in 7 patients.

Except for one patient who died prior to engraftment, all 40 other patients engrafted. Acute graft-versus-host disease developed in a total of 9 of 41 patients at risk. This included 1 of the 11 recipients of HLA-matched related transplants, and 4 of 16 recipients of HLA-matched unrelated transplants, and 4 of 13 recipients of HLA-mismatched unrelated or related transplants. Staging included grade 1 in 4 patients, grade 2 in 4 patients, grade 3 in 1 patient, and no grade 4. The overall cumulative incidence of grade 2-4 acute GvHD was 12%. There was no difference between the recipients of HLA-matched or mismatched donors. Chronic GvHD developed in 2 patients of 34 at risk. The overall cumulative incidence of chronic GvHD at one year was 5%. Thus, the overall incidence of engraftment and GvHD was quite safe

Of the 41 patients, 25 are alive, all in remission and no patients with relapsed disease. The overall survival (OS) and disease-free survival at 2 years post transplant for all patients were 64% and 57% respectively. For patients transplanted with minimal residual disease, the OS and DFS were 63% and 58% respectively, and for patients with persistent but limited disease 67% and 50%. For patients with primary MDS, the OS and DFS were 76% and 63% respectively and for patients with therapy-related MDS, 46% and 47%. For recipients of HLA-matched related donor transplants, the OS and DFS were 48% and 30% respectively, and for recipients of HLA-matched unrelated donor transplants 65% and 65%, and for recipients of HLA-mismatched transplants 69% and 58%.

Relapse occurred in a total of 4 patients; with a cumulative incidence at 2 years of 12%. One of the relapses occurred in 1 patient in CR (7%), 2 in patients with RA (10%), and 1 in a patient with RAEB-1/RAEB-2 (17%). Relapses occurred in 2 out of 25 patients (8%) with primary MDS and 2 out of 16 patients (12.5%) with therapy related MDS. Relapses occurred in 2 recipients of 28 HLA-matched transplants and 2 recipients of 13 HLA-mismatched transplants.

Non-relapse or transplant-related deaths occurred in 13 patients. The overall cumulative incidence of transplant-related mortality at one year post transplant was 22%. The CI of TRM by age was 17% for the 12 patients aged <50 years and 24% for the 29 patients aged $\geq$ 50 years. This difference was not statistically different. The CI of TRM by donor was 27% for recipients of matched related transplants and 20% for recipients of unrelated transplants. Also, non-relapse deaths occurred in 7 of 25 patients with primary MDS (28%) and 6 of 16 patients with secondary MDS (37%). Infections were the most common cause of death. Of the 13 deaths, 4 were due to an infection occurring within the first 3 months post transplant (3 patients) or thereafter (1 patients). All these patients had poor immune recovery as determined by low CD4 count and poor response to PHA.

In summary, this phase II 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. Transplant related deaths were the

main cause of transplant failure and the leading causes were infections, which resulted from the delayed recovery of immune function in these patients.

### *3.4. Palifermin*

Keratinocyte growth factor (KGF) is an endogenous protein in the fibroblast growth factor (FGF) family that binds to KGF receptor. Binding to its receptor has been reported to result in proliferation, differentiation, and migration of epithelial cells in many tissues including the buccal mucosa, esophagus, stomach, intestine, salivary gland, lung, liver, pancreas, kidney, bladder, mammary gland, skin, the lens of the eye, and the thymus. Hematopoietic cells are unresponsive to KGF as they lack its receptor.

Palifermin is a human keratinocyte growth factor produced by recombinant DNA technology in Escherichia coli. It differs from endogenous human KGF in that the first 23 N-terminal amino acids have been deleted to improve protein stability. In mice and rats Palifermin enhanced proliferation of epithelial cells and demonstrated an increase in tissue thickness of the tongue, buccal mucosa and gastrointestinal tract. Palifermin has been studied in murine models of chemotherapy and radiation-induced gastrointestinal injury. In such models, administration of Palifermin prior to and/or after the cytotoxic insult improved survival and reduced weight loss compared to control animals.

The toxicity and pharmacokinetics of Palifermin was studied in healthy subjects and patients with hematologic malignancies. After single IV doses of 20 to 250 mcg/kg (healthy subjects) and 60 mcg/kg (cancer patients) Palifermin concentrations declined rapidly (over 95% decrease) in the first 30 minutes per dose. The elimination half-life was similar between healthy subjects and cancer patients (average 45 hours with a range of 3.3 to 5.7 hours). A phase I trial indicated that Palifermin at doses up to 80 mcg/kg/d/3 consecutive days was not associated with major adverse events [57]. No accumulation of Palifermin occurred after 3 consecutive daily doses of 20 or 40 mcg/kg in healthy volunteers, or 60 mcg/kg in cancer patients.

The safety and efficacy of Palifermin were established in a randomized double-blind, placebo-controlled clinical study of 212 patients, who received high-dose cytotoxic therapy consisting of fractionated total-body irradiation (TBI) 12 Gy total dose), high dose etoposide (60 mg/kg) and high-dose cyclophosphamide (100mgkg) followed by peripheral blood progenitor cell (PBPC) support for the treatment of hematological malignancies (NHL, Hodgkin's disease, AML, ALL, CML, CLL, or multiple myeloma) [58]. Patients were randomized to receive either Palifermin (n = 106) or placebo (n = 106). Palifermin was administered as a daily IV injection of 60 mcg/kg for 3 consecutive days prior to initiation of cytotoxic therapy and for 3 consecutive days following infusion of PBPC. Compared with placebo-treated patients, Palifermin–treated patients reported less mouth and throat soreness. There was a reduction in median days of WHO Grade 3-4 oral mucositis (4 vs. 6 days), lower incidence of WHO Grade 3-4 oral mucositis (67% vs. 80%) and lower incidence of WHO Grade 4 oral mucositis (26% vs 50%). Palifermin was approved by the FDA for the prevention of chemotherapy and or radiation induced mucositis.

Palifermin has been also tested in the allogeneic HSCT setting for potential benefits on acute GvHD and hematopoietic reconstitution. The results of a phase 1-2 randomized, double-blind, placebo controlled dose-escalation trial of Palifermin in patients with hematologic malignancies conditioned with cyclophosphamide and fractionated total body irradiation or busulfan and cyclophosphamide was recently reported [59]. No significant differences in the incidence or severity of acute GvHD, or neutrophil and platelet engraftment was observed between patients

receiving or not receiving Palifermin. Palifermin was associated with reduced incidence and mean severity of mucositis in patients conditioned with cyclophosphamide and TBI.

KGF has been shown to play an important role in T cell homeostasis by its role in regulating the proliferation and differentiation of thymic epithelium. In murine models, KGF regulates thymic development, as it is required for postnatal thymic regeneration [60-61]. Also, in murine allogenic transplant models, KGF given before transplant has a protective effect on the thymus and thymopoiesis after transplantation [62 63]. A recent study in rhesus macaques also showed that Palifermin augments T cell immune reconstitution after autologous HCST (58b).

### *3.5. Summary*

Based on: (i) our previous trial of busulfan, melphalan, fludarabine, ATG and T cell depleted allogeneic SCT which showed a significant improvement in DFS for patients with myeloid malignancies, particularly in patients with advanced MDS and AML evolved from MDS. However, the major limitation to the success of these transplants in older patients and recipients of unrelated and HLA 1-2 allele disparate grafts in remission is the risk of the non-relapse mortality due to opportunistic infections which in these patients extends beyond the 6-14 months post transplant. (ii) observations in murine and primate models showing the beneficial effect on thymus and T cell lymphopoiesis; and safety and efficacy on mucositis and associated complications in patients undergoing autologous and allogeneic SCT. We propose this trial aimed at fostering earlier recovery of thymopoiesis and thereby to decrease the non-transplant morbidity and mortality and therefore to improve the overall and disease-free survival in patients with advanced MDS and AML evolved from MDS undergoing allogeneic transplantation from HLA-matched or mismatched related and unrelated donors. Patients will be evaluated for the incidence of opportunistic infections in the post transplant period, immune reconstitution, non-relapse mortality and morbidity and overall survival and disease free survival.

The success of allogeneic SCT in patients with advanced MDS depends on multiple variables, our approach involving induction of remission before cytoreduction for transplantation and then a T cell depleted myeloablative allogeneic transplant markedly reduces two major obstacles limiting the success of allogenic transplantation, disease relapse and graft versus host disease. This phase II trial incorporating KGF is designed to reduce the non-relapse mortality post transplant and to improve the DFS

## 4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

### 4.1 Design

This is a single arm phase II trial to assess the efficacy (decrease the transplant related mortality) and safety of peri-transplant Palifermin in combination with 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 advanced MDS and AML evolved from MDS. The addition of Palifermin is to decrease the toxicity and the infection rate associated with this regimen and transplant type and to foster earlier immune reconstitution.

### 4.2 Intervention

Patients will receive Palifermin 60 mcg/kg/day IV on three consecutive days with the last dose administered no less than 24 and no more than 48 hr prior to start of cytoreduction. The preparative regimen to be used for transplants will consist: of busulfan administered in 12 doses

over three days of 0.8 mg/kg IV for patients ≥ 4 years of age or 1.0 mg/kg IV for patients < 4 years of age; melphalan 70 mg/m$^2$ IV x 2 days; and, fludarabine 25 mg/m$^2$ IV x 5 days. Patients will receive ATG for two doses prior to transplant. G-CSF mobilized CD34+E- PBSCs (or BM if donors are unwilling or unable to donate PBSC) obtained from the HLA compatible donor will be infused on day 0. Patients will receive three additional daily doses of Palifermin, the first approximately 6 hours after the stem cell infusion on day 0, followed by two daily doses given on d+1 and d+2. Supportive care will be administered as per the BMT Service guidelines.

## 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.

5.5. *Palifermin*:

**a. Source and pharmacology:** Supplier: Amgen. Palifermin (Kepivance) is a human keratocyte growth factor produced by recombinant DNA technology in Escherichia coli. It differs from endogenous human KGF in that the first 23 N-terminal amino acids have been deleted to improve protein stability

**b. Formulation and stability:** Palifermin is supplied in vials containing 6.25 mg.

**c. Solution preparation:** The dispensed Palifermin vial contains lyophilized powder. It should be reconstituted with sterile water for injection, USP to yield a final concentration of 5 mg/ml.

**d. Storage and stability:** the lyophilized powder should be stored at 2 to 8 degrees centigrade and protected from light. The reconstituted solution should be also protected from light and administered immediately as it has no preservative.

**e. Administration:** The reconstituted solution should be administered immediately by intravenous bolus injection. If heparin is used to maintain the IV line, saline should be used to rinse the line prior to and after Palifermin administration since Palifermin has been shown to bind to heparin in vitro.

## 6.1 CRITERIA FOR SUBJECT ELIGIBILITY

Diagnosis (this includes de novo or treatment- related cases):

1. Myelodysplastic syndrome (MDS): RAEB-1 and RAEB-2.
2. Chronic myelomonocytic leukemia: CMML-1 and CMML-2.
3. Acute myelogenous leukemia (AML) evolved from MDS.

Status after chemotherapy and determining eligibility of the patient

1. In hematologic remission.
2. In second refractory cytopenia phase
3. With persistent but limited disease after chemotherapy.

***DEFINITIONS OF DISEASE STATUS***

The MDS subtype and prognostic classification at diagnosis and before transplantation will be determined according to the WHO (World Health Organization) and IPSS (International Prognostic Scoring System) criteria [64, 65].

**Complete remission after chemotherapy** is defined as a cellular marrow with <5% blasts with no overt dysplasia and blood count recovery [66, 67].

**Second refractory cytopenia after chemotherapy** is defined as a marrow with <5% blasts with or without dysplasia, and with persistent pancytopenia with no circulating blasts [15].

**Persistent but limited disease after chemotherapy**: <5% blasts in peripheral blood and 5-9% blasts in the bone marrow (similar to RAEB-1).

***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 is either matched at all A, B and DRB1 loci or who is at least 5/6 allele matched (HLA A, B and DR) and 8/10 allele matched (HLA A, B, C, DR, DQ) to the recipient will be eligible for entry on this protocol.

### 6.1 Subject inclusion criteria

- Patients should be < 65 years. Patients ≥ 65 years will be accrued on a case by case basis after discussion and approval by the BMT Service.
- Patients may be of either gender or any ethnic background.
- Patients must have a Karnofsky or Lansky 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 > 60-ml/min/1.73 $m^2$
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.
- Parent or legal guardians of patients who are minors will sign the informed consent form.

### 6.2 Subject exclusion criteria

- Active CNS or skin leukemic involvement
- 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 a prior allogeneic or autologous stem cell transplant within the previous six months.
- Patients who have had a previous malignancy that is not in remission.

## 7.0 RECRUITMENT PLAN

Eligible patients will be identified through the weekly BMT review meeting. Once transplant dates and collection dates are set, they will be offered this trial and informed of the rationale for this trial, as well as the logistic implications and the risks and benefits. This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations.

## 8.0 PRETREATMENT EVALUATION

### 8.1. Pretreatment evaluation of the patient

The patient will receive an extensive medical evaluation approximately 45 days prior to starting preparatory cytoreduction. This evaluation will include the below tests:

- Physical exam and medical history

- Dental evaluation (may be completed outside of the 45 day window)
- CBC
- Coagulation profile
- Blood Type and screen
- Serum chemistries including BUN, creatinine, electrolytes, glucose, total protein, albumin, liver function tests (AST, ALT, bilirubin, alkaline phosphatase).
- Serum will be tested for CMV, Herpes Zoster, Herpes Simplex, Hepatitis B, Hepatitis C, EBV, Syphilis (adolescents and adults), Measles (pediatric patients only) and Toxoplasmosis.
- Blood will also be tested for HTLV-1 and 2 as well as HIV-1 and 2, and West Nile
- Pregnancy test for women of childbearing age
- Bone marrow aspirate and biopsy
- Urinalysis
- Electrocardiogram, echocardiogram or a gated pool scan if needed
- Pulmonary function test if needed
- Chest X-ray or other types of scans (i.e. CT scan or PET scan) as needed
- Samples of bone marrow and/or peripheral blood cells will be obtained to define donor/host differences and to determine engraftment of donor cells. (may be completed outside of 45 day window)
- An additional 6 tablespoons of blood will be drawn for laboratory tests during routine blood tests if needed

## 9.0 TREATMENT/INTERVENTION PLAN

### 9.1. ***Preparative cytoreduction***

Patients will be treated as inpatient on the Memorial Hospital Allogeneic Transplant Services in Pediatrics or Medicine. The patient will have a central venous catheter present for the transplant. Once neutropenic, the patient will be maintained in reverse isolation until the day of discharge.

***The preparative cytoreduction will include the following:***

Days –9 to –7 **Busulfan* 0.8 mg/Kg/dose Q6H X 12 doses/3 days IV**

If ≥ 4 years of age over 2 hours

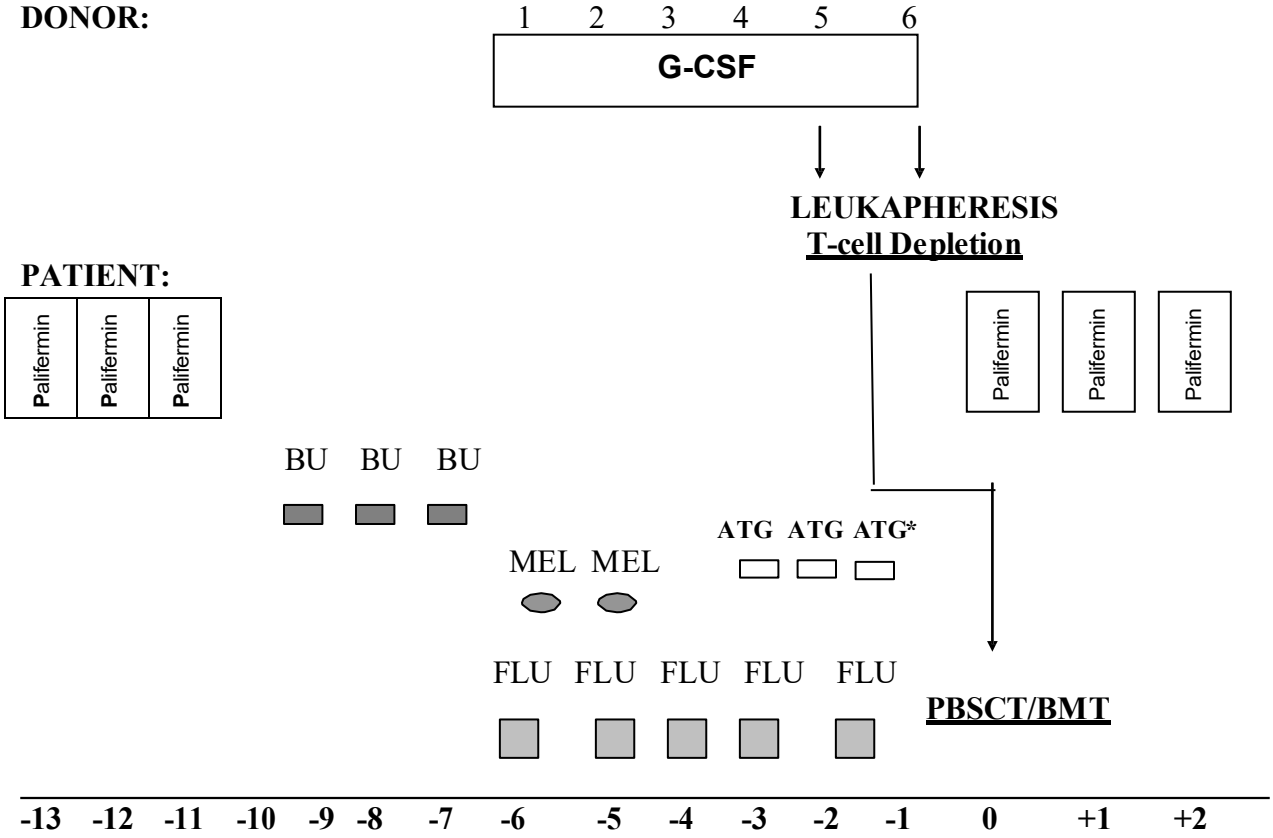
**(Busulfex®) 1.0 mg/Kg/dose Q6H X 12 doses/3 days IV**

If < 4 years of age over 2 hours

Days -6 to -5 **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***




*Last dose of ATG is given to recipients of HLA-mismatched stem cell transplants ONLY.

## ***AN EXAMPLE OF A ROAD MAP OF PREPARATION FOR TRANSPLANT***

| SUNDAY | MONDAY | TUESDAY | WEDNESDAY | THURSDAY | FRIDAY | SATURDAY |
|---|---|---|---|---|---|---|
| | | | | | -13<br>**Palifermin**<br>60 mcg/kg/dose<br>IV X 1 | -12<br>**Palifermin**<br>60 mcg/kg/dose<br>IV X 1 |
| -11<br>**Palifermin**<br>60 mcg/kg/dose<br>IV X 1 | -10 | -9<br>**Busulfan** IV<br>0.8-1.0<br>mg/Kg/dose<br>IV Q 6H X4 | -8<br>**Busulfan** IV<br>0.8-1.0<br>mg/Kg/dose<br>IV Q 6H X4<br><br>Busulfan dose will be adjusted per PK levels when available | -7<br>**Busulfan** IV<br>dose per PK<br>IV Q 6H X2<br>**Busulfan** IV<br>dose per PK<br>IV Q 6H X2 | **-6**<br>**Fludarabine**<br>25 mg/m2/day<br>IV X 1<br><br>**Melphalan**<br>70 mg/m2/day<br>IV X 1 | -5<br>**Fludarabine**<br>25 mg/m2/day<br>IV X 1<br><br>**Melphalan**<br>70 mg/m2/day<br>IV X 1 |
| -4<br>**Fludarabine**<br>25 mg/m2/day<br>IV X 1 | -3<br>**Fludarabine**<br>25 mg/m2/day<br>IV X 1<br><br>**Rabbit ATG**<br>2.5 mg/Kg/d<br>IV over 12 H | -2<br>**Fludarabine**<br>25 mg/m2/day<br>IV X 1<br><br>**Rabbit ATG**<br>2.5 mg/Kg/d<br>IV over 12 H | -1<br><br>**Rabbit ATG**<br>2.5 mg/Kg/d<br>IV over 12 H for HLA – mismatched SCTs only | 0<br>**T-cell depleted Stem cell transplant**<br><br>**Palifermin**<br>60 mcg/kg/dose<br>IV X 1 | **+ 1**<br><br>**Palifermin**<br>60 mcg/kg/dose<br>IV X | +2<br><br>**Palifermin**<br>60 mcg/kg/dose<br>IV |
| +3 | **+4** | +5 | +6 | +7<br>Start **G-CSF** | | |

Schedule may be adjusted for patient safety or donor scheduling issues, if unavoidable.

**** busulfan dosing and administration:***

**Patients will have busulfan levels drawn whenever possible after the first dose on day 1, with adjustments in dosing based on the pharmacokinetics of the first dose according** to "institutional" standard clinical practice as indicated. 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.

***** 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 administered is based on the patient's actual weight per MSKCC standard of care guidelines.

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

9.2. *Palifermin*: will be administered intravenously to all patients on day -13, -12, -11 and 0, +1, +2. Dose will be calculated using actual body weight.

9.3. *Peri-transplant treatment to promote engraftment*

**a. Rabbit anti-thymocyte globulin* (Thymoglobulin®) and methylprednisolone (MPD)**
Rabbit ATG will be given to all transplant recipients > 18 years old. Rabbit ATG will also be given to patients ≤ 18 years of age who receive transplants from donors other than HLA-matched related siblings. Patients will receive rabbit ATG (Thymoglobulin®) at 2.5 mg/Kg/day x 2 days on days -3 and -2. Recipients of HLA-mismatched stem cell transplants will receive an additional dose of rabbit ATG on day -1. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 2 or 30 mg/kg x 1 may be used. An additional dose of equine ATG will be given to recipients of HLA-mismatched stem cell transplants. If severe reaction is encountered after the first dose of ATG, the second dose can be delayed until day +5. Methylprednisolone will be given at 1 mg/Kg/day with the ATG administration and will be discontinued thereafter**.** If patient is receiving a second transplant from the same donor, ATG administration will be at the discretion of the physician. ATG dosing should be adjusted if patient is > 125% of ideal body weight and should be calculated on adjusted ideal body weight per MSKCC standard of care guidelines.

Patients may receive premedication consisting of acetaminophen, diphenhydramine, ranitidine, and an additional dose of methylprednisolone (1 mg/kg/dose)

**b.** G-CSF will be given to all patients, regardless of transplant type, from approximately Day +7 if absolute neutrophil count (ANC) is <500 and continue until ANC is ≥2000. G-CSF may be given earlier post transplant if clinically indicated at the discretion of the Attending Physician. G-CSF doses will be given and rounded according to Adult and Pediatric BMT guidelines.

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

No further GvHD prophylaxis will be administered.

9.5. *Stem Cell Transplantation*

*9.5.1.* ***PBSCT***

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

For related donors, ideally 5-6 days before the day of transplant, the donor will receive GCSF per institutional guidelines. On approximately the fifth and sixth days of this course of G-CSF, the donor will undergo leukaphereses designed to provide a minimum of $10^9$ mononuclear cells/kg of the transplant recipient's weight. For unrelated donors, G-CSF will be administered and the leukaphereses obtained according to the National Marrow Donor Program protocol IND, and institutional guidelines. Mononuclear cell fractions collected on the fourth and fifth days will be pooled.

**The CliniMACS System for Positive Selection of CD34+ Progenitor Cells and Depletion of T-Cells.**

The CliniMACS System (Miltenyi Biotec, Auburn, CA) including the CliniMACS$^{plus}$ Instrument, a CliniMACS Tubing Set, the CliniMACS CD34 Reagent and the CliniMACS PBS/EDTA Buffer is intended for the selection and enrichment of human CD34 positive hematopoietic progenitor cells from a leukapheresis product.

The CD34 antigen is a cell membrane glycoprotein expressed by early hematopoietic stem and progenitor cells. The CD34 positive cell separation process may be useful in several areas of clinical stem cell transplantation, including purging of tumor cells, T-cell depletion, *ex vivo* cell expansion and gene therapy. When re-infused after myeloablative chemotherapy, CD34 positive peripheral blood progenitor cells have been shown to reconstitute all hematologic lineages and exhibit both short and long term repopulating capacities.

The ClinMACS System uses selective CD34 monoclonal antibodies conjugated to super-paramagnetic particles. The CD34 positive target cells are selected in an automated continuous flow separation system.

The CD34 positive cells are specifically labeled for selection by incubation with the CliniMACS CD34 Reagent. After unbound reagent is washed from the suspension, the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension, the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension through a column in which strong magnetic gradients are generated. The Selection Column retains the magnetically labeled CD34 positive cells, while unwanted cells flow through the Selection Column and are collected in the Negative Fraction Bag. The system performs several washing steps, disposing most of the liquid into the Buffer Waste Bag. The Separated CD34 positive cells are released from the column by removing the column from the magnetic field and eluting the cells into the Cell Collection Bag.

The components of the CliniMACS System include:

**The CliniMACS Instrument**
The CliniMACS Instrument is a bench-top instrument consisting of a supporting structure to hold the column/tubing assembly and various bags, a series of valves through which the tubing set is fitted, a magnet between the poles of which the separation column is placed, a peristaltic pump through which a section of tubing is placed, software to control the instrument and user interface and a computer touchpad with a display window. The instrument is operated at ambient temperature and it is intended to be multi-use item.

The software for the CliniMACS Instrument controls the function of the electromechanical components of the instrument and the user interface. Two separate computers, one a micro-controller located on a control board of the CliniMACS Instrument and the second a PC compatible computer which operates the user interface are incorporated with the instrument. Software Version 2.31, the current version of software is directly traceable to the version of software utilized in pre-clinical testing and European Safety trials, and has been inspected and approved by TÜV product services with the CE Mark.

**CliniMACS Tubing Set**
The CliniMACS Tubing Set consists of a tubing element combined with a pair of proprietary cell selection columns. These form a closed, sterile system for processing the cells. The separation column is a proprietary component of the CliniMACS System consisting of a plastic column housing with polypropylene frits in each end. The interior of the column housing is filled with a matrix of sub-millimeter iron beads coated with a heat-cured biocompatible resin. The columns are placed at appropriate locations in the CliniMACS Tubing Set to facilitate the cell selection process. The first column serves as a device to remove components that bind non-specifically to the column. The second column which is placed within a magnetic field performs the actual cell selection. The columns are incorporated sterile as part of the tubing set and are intended for single use only.

The tubing element consists of a series of tubes, connectors, spikes, Luer locks, and collection bags. The tubing of the tubing element is comprised of materials that have been qualified for use in this application by testing to ISO 10993. The principal constituents are polyvinyl chloride (PVC) and silicone. The connectors are made of various polymers (e.g., ABS and PVC) suitable for use in a blood contact environment. They are solvent bonded to the PVC tubing. The silicone pump tubing is softened with petroleum ether for manufacturing and mechanically fixed to connectors. The cell wash bags are composed of PVC.
The CliniMACS Tubing Set is packaged in a thermoformed tray and heat sealed with a Tyvek® lid. The CliniMACS Tubing Set is sterilized by ethylene oxide gas in a validated sterilization cycle and supplied as a single-use component for the CliniMACS Instrument.

**CliniMACS CD34 Reagent**
The CliniMACS CD34 Reagent is a dark amber, nonviscous, colloidal solution containing the antibody conjugate in buffer. The conjugate consists of a monoclonal antibody towards the human CD34 antigen. The murine monoclonal IgG1 antibody is covalently linked to dextran beads having an iron oxide/hydroxide core. The concentration of the conjugate is equivalent to 20 micrograms (µg) per mL of antibody protein, 800 µg/mL of dextran and 800 µg/mL of iron. The colloid is buffered in a phosphate-buffered saline (PBS) containing ethylenediaminetetraacetic acid (EDTA) and Poloxamer 188. The nominal concentrations of its components are 0.0095 M phosphate, 0.004 M potassium, 0.163 M sodium, 0.139 M chloride, 0.005 M EDTA and 0.03 % (w/v) Poloxamer 188. The pH is 7.4 - 7.7. Poloxamer 188 is added to the CliniMACS CD34 Reagent to stabilize it during shipping, handling and storage. The CliniMACS CD34 Reagent is supplied sterile and pyrogen-free in glass vials containing 7.5 mL and is intended for single use and in vitro use only.

**The CliniMACS PBS/EDTA Buffer**
The CliniMACS PBS/EDTA Buffer is an isotonic and isohydric buffer solution with a pH-value of 7.2 and osmolarity of 290 mosmol/L. Its formulation is shown in the following table.

Table 1 Formulations of the CliniMACS PBS/EDTA Buffer

| Ingredient | Compendial | Amount |
|---|---|---|
| NaCl | Ph. Eur. | 8.0 g/L |
| KCl | Ph. Eur. | 0.19 g/L |
| Na2HPO4 anhy. | Ph. Eur. | 1.15 g/L |
| KH2PO4 | Ph. Eur. | 0.19 g/L |
| Na2EDTA | Ph. Eur. | 0.37 g/L |
| Water for Injection | Ph. Eur. | ad 1L |

The CliniMACS PBS/EDTA Buffer is used as external wash and transport fluid for the in vitro preparation of human heterogeneous cell populations intended to be separated with the CliniMACS Cell Selection System. 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.

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.

Throughout the process, critical control points and associated assays are identified and performed on each cellular therapy product. Aliquots of the same product sample are taken for in-process and final product testing. After each step (apheresis, platelet washing, CD34 labeling and washing, enrichment), QC testing includes; Sterility. Endotoxin, gram stain, Total nucleated cells (TNC), flow cytometry phenotype/analysis of CD45, CD34, and CD3, and viability assessed by 7-AAD is assessed. To ensure sterility, 14-day sterility tests are performed for in-process sterility testing and a gram stain is performed on the final product prior to release.

*9.5.2.* ***BMT (if PBCST not possible)***

Approximately 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 [38, 39] and the SOP from the MSKCC Cytotherapy Lab is appended.

The amount of marrow harvested will be such to provide a minimum of 1 x $10^7$ 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 sRBC 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.***

Ideally, 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 approximately 15 minutes with monitoring of vital signs. The patient is premedicated as for blood product transfusions.

9.6. *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***

Post-pubertal females may receive prophylaxis against menorrhagia according to our standard of care guidelines**.**

**c.** ***Transfusions***

Following initiation of the pre-transplant cytoreduction, all blood products for transfusion, with the exception of the marrow graft, will be irradiated to 3,000 cGy to inactivate lymphocytes capable of initiating lethal GvHD. Blood products are irradiated in the blood bank, using a cesium gamma emitter. Patients who are Cytomegalovirus (CMV)-seronegative

pre-transplant will be permitted to receive only CMV-seronegative blood products, except in an emergency.

Platelets may be administered for clinical evidence of active hemorrhage. To minimize bleeding, platelets will be transfused prophylactically in order to maintain a platelet count greater than 20,000/mm$^3$. Post-transplantation, the marrow donor or selected family donors will be utilized, whenever possible, as platelet donors. Packed irradiated red blood cells will e administered as clinically indicated.

**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.

## 10.1 EVALUATION DURING TREATMENT/INTERVENTION

### 10.2 Post-transplant evaluation

The chart below shows the approximate dates for tests and procedures performed after transplant. Certain tests may be held at the discretion of the treating physician and/or if deemed in the best clinical interest of the patient.

| ACTIVITY | TRANSPLANT TO DISCHARGE | DISCHARGE TO DAY 100 | DAY 100 - 6 MONTHS | LONG TERM FOLLOW-UP |
|---|---|---|---|---|
| Blood counts and chemistry (CBC, Comprehensive Metabolic Panel) | CBC: daily<br>Comp: 2 times per week | CBC, Comp: weekly, or twice a week or every 2-3 weeks as needed | Every 2-4 weeks | 9, 12 & 24 months after transplant; thereafter as clinically indicated |
| Physical exam for GVHD evaluation | Daily after engraftment until discharge from the hospital | About weekly after discharge until day 100 | Every 2-4 weeks | 9, 12 & 24 months after transplant; thereafter as clinically indicated |
| Disease evaluation, Bone marrow, & peripheral blood chimerism by cytogenetic tests | 28-30 days after transplant | 100 days after transplant | 6 months after transplant | 9 months (if clinically indicated) 12 months, 18 months (if clinically indicated) & 24 months after transplant; thereafter as clinically indicated |
| Peripheral blood lymphocyte (PBL) phenotyping | NA | 90-120 days after transplant* | | 9 months after transplant; thereafter every 3 months if clinically indicated |
| In vitro response of PBL to standard panel of mitogens, antigens, and | NA | NA | 6 months after transplant | thereafter every 3-6 months until normal or plateau |

| | | | | |
|---|---|---|---|---|
| viral antigens | | | | |
| Research Blood Tests | 15, 28 days after transplant | 60 and 100 days after transplant | 6 months after transplant | 12 months after transplant |

Tests may be done more frequently if there are complications such as GvHD or infections.
** PBL phenotyping is part of standard of care; therefore it is billable to the patient or insurers.*

**10.3 Research Samples** (as listed in the chart above)
Mononuclear cells (obtained from approximately 20-30cc of whole blood) may be obtained from the patient prior to transplant, as well as at approximately the same time points chimerism samples are obtained. These cells will be studied for alloreactivity and antitumor reactivity in vitro. Research blood tests will be performed at the discretion of the physician.

## 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 GCSF is frequently associated with low grade fever and low back pain which usually resolves within one day following cessation of GCSF treatment. Furthermore, there has now been one recorded patient who developed acute splenomegaly and splenic rupture in response to high dose GCSF. 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, leukapheresis 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 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.

**Melphalan:** 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, 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.

**Palifermin:** skin rash, itching, redness, swelling, skin sensitivity, muscle and joint aches, tingling in the mouth and lips, mouth/tongue thickness or discoloration, abnormal taste. Skin rash can be treated with anti-histamine medications and pain with Tylenol. The other symptoms resolve with no medications.

**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.

**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.

**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 skin 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.

**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.

**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.

**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:**

**Risk of a secondary cancer** different from MDS/AML 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 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.

**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.

**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.

**Recurrence of MDS/AML** is a risk even if the transplant is initially successful.

## 12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

**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 Palifermin).

**Transplant-related mortality** (TRM) includes the RRT and other fatal complications resulting from the allogenic transplant such as graft failure, GvHD, hemorrhages, and infections.

The grading for monitoring the morbidity and mortality will be based on the NCI/CTEP common toxicity criteria.

### 12.2. Infections.

The occurrence of life-threatening opportunistic infections will be evaluated according to the criteria established by BMT CTN (see Appendix) and will correlate this with the level of immune recovery. The infection-related mortality will be also determined.

### 12.3. 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 may be evaluated more frequently, as graft rejection, late graft failures, more pronounced immune dysfunction, and more patient to patient variability have been observed.

Immunophenotyping and NK cell function will be performed on circulating lymphocytes of all recipients of T-cell depleted marrow transplants during the first post-transplant month to monitor engraftment. Lack of NK cells and NK function and predominance of $CD8^+$ host cells have correlated with impending graft failure. Thereafter, immunophenotyping of T-cells, B-cells, and NK cells, will be performed at approximately 1, 3, and every 3-6 months post-transplant until normal values are reached. The approximate schedule tests are shown in the table on page 23.

T-cell proliferations in response to PHA, Candida, viral antigens, and tetanus following immunization will be performed at approximately 6 months post-transplant and every 3-6 months thereafter until normal values are reached

Immunoglobulin levels will be tested at 6, 12, and 24 months post-transplant and thereafter as clinically indicated. Patients with normal total IgG levels and PHA response within the 10th percentile of normal may be re-immunized according to infection guidelines.

### 12.4 Engraftment and chimerism

Engraftment will be documented by analysis of T cells and bone marrow cells for chimerism by standard cytogenetic studies at about 1 month, 100 days, 6 months, 9 months (if clinically indicated), and 12 months, 18 months (if clinically indicated), and 24 months post transplant or as needed thereafter.

### 12.5 Graft failure or rejection

T cell depletion of donor cells is associated with an increased incidence of graft failure in allogeneic transplant recipients. After allogeneic transplantation, the recipient's marrow function may be poor and leukopenia, anemia, or thrombocytopenia may result from many causes including graft rejection induced by surviving host immune T-cells, or ongoing suppression of engrafted donor blood-forming cells by GVHD, infection or marrow suppression or immunosuppressive drugs and other medications. Graft failure may result in death if not reversed. In patients with immune rejection second transplants can be administered with immunosuppressive therapy, including non-myeloablative conditioning regimens. For patients who are engrafted with donor cells but have severe cytopenia affecting one or more blood cell lineages, secondary transplants of CliniMACS fractionated CD34+ T-cell depleted PBSCs may be administered to booster and replenish donor hematopoietic cells without conditioning or after treatment with anti- thymocyte globulin.

### 12.6. Graft-versus-host disease

Standard BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al [68] (see appendix 2) 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. Data collection may 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 as long as they have not received donor derived leukocytes infusions (DLI) for the treatment of relapse or infections. If at any time, a patient receives DLI, that time will represent the end-time for evaluation of 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 (2-20mg/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.

Late acute GvHD and Chronic GvHD will be defined according to the criteria of National Institutes of Health consensus criteria[69] treated with standard or experimental immunosuppressive therapy. Treatment may 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).

### 12.7. Disease relapse

Relapse of MDS or AML will be analyzed as to type and genetic origin of the leukemic cells. These will be defined by an increasing number of blasts in the marrow over 5%, by the presence of circulating peripheral blasts, or by the presence of blasts in any extramedullary site. Cytogenetic analysis of the marrow and/or peripheral blood will also be obtained for the diagnosis of relapse.

## 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**.** Patients may also be removed from the study at any point deemed appropriate by the principle investigator.

## 14.1 BIOSTATISTICS

This is a revision of a phase 2 trial designed to investigate the efficacy and safety of peri-transplant Palifermin in combination with 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 advanced MDS and AML evolved from MDS. This amendment is in response to excessive graft failure in patients with mismatched grafts. As a result, the study population will be divided into two groups: HLA matched and HLA mismatched donors. The data analysis and data monitoring will conducted separately in each HLA group. A maximum of 31 patients in the HLA matched donor group and 21 patients in the HLA mismatched donor group are planned for accrual onto the study. It is anticipated that accrual will last three years and each patient will be followed for a minimum of

two years if he/she has not failed. The primary endpoint of the study is one-year treatment related mortality (TRM).

After the first 16 patients were enrolled on this study, the transplant team altered the study design to make the treatment more effective and reduce the relapse rate and the rejection rate by changing the following:

1. Increasing the number of Busulfan doses from 10 to 12 doses
2. Increasing the number of ATG doses for recipients of HLA-mismatched transplants from 2 to 3 doses

As a result of these changes to the study design, accrual for the study restarted from zero as of the Amendment 5 approval, 10/15/2009.

For patients in the HLA matched donor group, a study design that differentiates between treatment related mortality probabilities of 0.10 and 0.30 will be used to assess treatment efficacy. At the conclusion of the study, if at least 25/31 patients do not experience treatment related mortality within the first year of transplant, the treatment will be declared a success in controlling treatment related mortality. The probability of declaring the treatment a success for TRM is 0.09 when the one-year TRM in the population is 0.30 and increases to 0.90 when the one-year TRM in the population is 0.10.

For patients in the HLA mismatched donor group, a study design that differentiates between treatment related mortality probabilities of 0.10 and 0.35 will be used to assess treatment efficacy. At the conclusion of the study, if at least 17/21 patients do not experience treatment related mortality within the first year of transplant, the treatment will be declared a success in controlling treatment related mortality. The probability of declaring the treatment a success for TRM is 0.10 when the one-year TRM in the population is 0.33 and increases to 0.89 when the one-year TRM in the population is 0.10.

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. The stopping rules for excessive failure and the corresponding power calculations are derived separately for the matched and mismatched groups and are given in the tables below. In the event that the stopping boundary is crossed for one of the donor groups, the study will continue accrual in the other group.

**HLA matched donor transplants (n=31)**

| Failure type | # of failures needed to stop the study | Probability of failure type | Probability of study completion |
|---|---|---|---|
| One-year TRM | 2 within the first 3 patients<br>3 within the first 8 patients<br>4 within the first 13 patients<br>5 within the first 19 patients<br>6 within the first 26 patients<br>7 in 31 patients | 0.30 | 0.09 |
| | | 0.10 | 0.90 |
| | 2 within the first 7 patients | 0.21 | 0.09 |

| | | | |
|---|---|---|---|
| Graft failure | 3 within the first 17 patients<br>4 within the first 29 patients<br>5 in 31 patients | 0.05 | 0.90 |
| Acute GvHD (grades 3-4) | 2 within the first 3 patients<br>3 within the first 8 patients<br>4 within the first 13 patients<br>5 within the first 19 patients<br>6 within the first 26 patients<br>7 in 31 patients | 0.30 | 0.09 |
| | | 0.10 | 0.90 |
| Chronic GvHD (extensive) | 2 within the first 3 patients<br>3 within the first 8 patients<br>4 within the first 13 patients<br>5 within the first 19 patients<br>6 within the first 26 patients<br>7 in 31 patients | 0.30 | 0.09 |
| | | 0.10 | 0.90 |

**HLA mismatched donor transplant (n=21)**

| Failure type | # of failures needed to stop the study | Probability of failure type | Probability of study completion |
|---|---|---|---|
| One-year TRM | 2 within the first 4 patients<br>3 within the first 8 patients<br>4 within the first 14 patients<br>5 in 21 patients | 0.33 | 0.10 |
| | | 0.10 | 0.89 |
| Graft failure | 2 within the first 4 patients<br>3 within the first 8 patients<br>4 within the first 14 patients<br>5 in 21 patients | 0.33 | 0.10 |
| | | 0.10 | 0.89 |
| Acute GvHD (grades 3-4) | 2 within the first 4 patients<br>3 within the first 8 patients<br>4 within the first 14 patients<br>5 in 21 patients | 0.33 | 0.10 |
| | | 0.10 | 0.89 |
| Chronic GvHD (extensive) | 2 within the first 4 patients<br>3 within the first 8 patients<br>4 within the first 14 patients<br>5 in 21 patients | 0.33 | 0.10 |
| | | 0.10 | 0.89 |

Kaplan-Meier estimates of overall and disease-free survival will be computed for the entire cohort and for the following subgroups: minimal residual vs. extensive disease, matched donor

vs. mismatched donor, primary vs. secondary MDS, age <50 vs. age >50. The accuracy of the estimates within these subgroups will be a function of the patient distribution. Estimated probabilities for the time to treatment related mortality, the time to infectious complication, and the time to graft versus host disease will be computed using the cumulative incidence function.

Immunologic reconstitution will be summarized by averaging the recorded results across patients at each time point. Graphic descriptions of the trajectories over time will be produced using kernel smoothing. To assess whether immune reconstitution is associated with demographic or transplant related variables, marginal regression models, based on generalized estimating equations, will be developed.

## 15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

### 15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

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.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

## 16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into the Clinical Research Data Base (CRDB), a secure database. Source documentation will be available to support the computerized patient record.

## 16.2 Quality Assurance

Weeklyregistration 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 on an ongoing basis.

### 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 Caner 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

Risks: From the studies that have been done so far there appears to be no increase in risk of addition of Palifermin. However, given this is a new treatment, it is possible that there are side effects that have not yet been seen.

Benefits: The information from this study will help future cancer patients.

Possible toxicities/side effects: Toxicities and side effects of the agents used are listed in section 11 and reporting of serious adverse events is found in section 17.2.

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.

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.

### 17.3 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- 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
- 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 information:
  - 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

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

For IND/IDE protocols:

The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

### 17.2.1 Definition of SAE

**An SAE** is an undesirable experience that meets one of the following criteria:

- Is fatal or life-threatening
- Is disabling
- Results in hospitalization or prolongation of hospitalization
- Results in congenital anomaly or occurrence of malignancy
- Important medical event that jeopardizes the participant AND requires medical or surgical intervention to prevent one of the outcomes above. *Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.*

**Attribution:**

- Unrelated: The AE *is clearly NOT related* to the intervention
- Unlikely: The AE *is doubtfully related* to the intervention.
- Possible: The AE *may be related* to the intervention.
- Probably: The AE *is likely related* to the intervention.
- Definite: The AE *is clearly related* to the intervention.

**Expected and Unexpected Event:**

- Expected: Any experience *previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan
- Unexpected: Any experience *not previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan

**UNEXPECTED EVENT:**

- Grades 1-2: Adverse Event Reporting NOT required.
- Grades 3: Possible, Probable, or Definite attribution to the drug and/or device will be reported*.
- Grades 4 and 5: Regardless of Attribution will be reported*.

**EXPECTED EVENT**

- Grades 1 – 3: Adverse Event Reporting NOT required.
- Grades 4 and 5: Regardless of Attribution will be reported*.

*Reportable events are those wich occur within 30 days of last dose of treatment on protocol. Events beyond 30 days will be reported at the discretion of the PI.

## 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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