**Abbreviated Title:** NK Cell Therapy

**NCI Protocol #:** 11-C-0073 R

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**Title:** A Phase I Study of NK Cell Infusion Following Allogeneic Peripheral Blood Stem Cell Transplantation from Related or Matched Unrelated Donors in Pediatric Patients with Hematologic Malignancies

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**Investigational Agents:**

| | |
|---|---|
| Drug Name: | Ex vivo Expanded Allogeneic NK Cells<br>KT64.4-1BBL Artificial Antigen Presenting Cells<br>rhIL15<br>Miltenyi CliniMACS® System |
| IND Number: | 14402 |
| Sponsor: | Center for Cancer Research, NCI |
| Manufacturer: | Cell Processing Department, Department of Transfusion Medicine, Clinical Center, NIH |

**Commerical Agents:** fludarabine, cyclophosphamide, etoposide, doxorubicin, vincristine, prednisone, cytarabine, methotrexate, busulfan, cyclosporine A, tacrolimus

## PRECIS

### Background

- Despite progress in pediatric oncology, some patient subsets with hematologic malignancies continue to experience extremely poor overall survival. Allogeneic Hematopoietic Stem Cell Transplant (HSCT) is effective in some high-risk hematologic malignancies.
- Allogeneic HSCT can be performed safely in these patient populations, but disease recurrence is common and new approaches to enhance the antitumor effect of this therapy are needed. NK mediated killing appears to confer improved outcomes after HSCT for patients with AML and ALL, and NK cell infusions have induced complete remissions in patients with AML.
- Preclinical data demonstrates that activated NK cells readily kill pediatric leukemias, that large numbers of activated NK cells can be generated *ex vivo* using artificial APCs and that the post-transplant period may be favorable for expansion and survival of adoptively transferred NK cells.

### Objectives

- To assess the feasibility and toxicity of infusing escalating doses of donor-derived activated NK cell donor lymphocyte infusions (NK-DLI) on Days 21 ± 3 days and 49 ± 7 days following HLA-matched, T cell depleted (TCD) PBSCT in patients with high risk leukemias who have unrelated donors or related donors
- To determine if patients treated in this manner experience rapid, sustained donor engraftment and acceptable rates of aGVHD (<25% incidence of grade III or grade IV).

### Eligibility

- Patients 4-35 years with hematologic malignancies (e.g., ALL, AML, CML, HD, NHL), with a 5/6 or 6/6 HLA-matched related or 9/10 or 10/10 HLA matched unrelated donor.

### Design

- Pre-transplant disease specific immune depleting chemotherapy and the preparative regimen will be the same as that used previously on 02-C-0259 and 01-C-0125, for those patients undergoing reduced intensity transplant.
- For patients with hematologic malignancies, a myeloblative regimen based on current Children's Oncology Group (COG) standard-of-care preparative regimen will also be included.
- Donors will undergo apheresis for filgrastim mobilized PBSC. This product will be T cell and NK cell depleted prior to cryopreservation. NK cells selected from the product will be used for *ex vivo* activation and expansion using KT64.4-BBL artificial antigen presenting cells.
- A phase 1 cell dose escalation of donor derived NK-DLI will be performed using 3 dose levels ($1 \times 10^5$, $1 \times 10^6$ and $1 \times 10^7$ NK cells/kg) infused on days 21±3 post-PBSCT and a second infusion on day 49 ± 7 post-PBSCT.
- Three patients will be enrolled at each dose level, with the cohort expanded to 6 if dose-limiting toxicity occurs. An expanded group of 12 patients will be treated at the highest dose level tolerated.

## TREATMENT SCHEMA

### Donor

**Stem Cell Harvest and NK Cell Collection**

Filgrastim mobilization, for 5-7 days until collection completed

Stem cell collection, target >10 x $10^6$ CD34 cells/kg, minimal 8 x $10^6$ CD34 cells/kg (pre-selection)

Stem cell cryopreservation and NK cell expansion

### Patient

**Fludarabine-EPOCH Induction Chemotherapy for Lymphoma or Leukemia Patients**

**1-3 cycles; 21 – 28 day cycles; Cycles 2 and 3 may be dose-modified as outlined in the protocol**

Fludarabine, 25 mg/$m^2$ per day IV over 30 minutes daily for 3 days; days 1-3

Etoposide, 50 mg/$m^2$ per day by continuous IV infusion daily for 4 days; days 1-4

Doxorubicin, 10 mg/$m^2$ per day by continuous IV infusion daily for 4 days; days 1-4

Vincristine, 0.4 mg/$m^2$ per day by continuous IV infusion daily for 4 days; days 1-4

Cyclophosphamide, 750 mg/$m^2$ IV over 30 minutes; day 5

Prednisone, 60 mg/$m^2$ per day in 2-4 divided doses PO for 5 days; days 1-5

Filgrastim, 5 μg/kg per day SQ from day 6 until ANC >1000/μl x 2 days

**FLAG Induction Chemotherapy for Leukemias (Non-myeloablative)**

**1-3 cycles; 21 – 28 d cycles; Cycles 2 and 3 may dose-modified as outlined in the protocol**

Filgrastim, 5 μg/kg per day SQ from day 0 until ANC >1000/μl x 2 days

Fludarabine, 25 mg/$m^2$ per day IV over 30 minutes daily for 5 days; days 1-5

Cytarabine, 2000 mg/$m^2$ IV over 4 hours daily for 5 days; days 1-5; Begin each dose 3.5 hours after completing fludarabine; Conjunctivitis prophylaxis with corticosteroid ophthalmic drops 2 drops to each eye every 6 hours starting prior to and continuing until 24 hours after completion of cytarabine.

**Pre-BMT Preparative Regimen (Non-myeloablative)**

Transplant Days –5 to -2

Fludarabine, 30 mg/$m^2$ per day IV over 30 minutes daily for 4 days; days –5, -4, -3, -2 of transplant

Cyclophosphamide, 1200 mg/$m^2$ per day IV over 2 hours daily for 4 days; days –5, -4, -3, -2 of transplant

Mesna, 1200 mg/$m^2$ per day by continuous IV infusion daily for 4 days; days –5, -4, -3, -2.

**Pre-BMT Preparative Regimen (Myeloablative)**

AML Patients:

| Day | Treatment |
|---|---|
| -6 | Anti Seizure Prophylaxis* |
| -5 to -2 | Fludarabine 40 mg/m$^2$/dose IV daily (1.3 mg/kg if <10kg)** |
| -5 to -2 | Busulfan 3.2 mg/kg/dose IV daily x 4 doses*** |
| -1 | Rest (an additional day may be added) |
| 0 | HSCT Infusion |

**Pre-BMT Preparative Regimen (Myeloablative)**

ALL Patients:

| Day | Treatment |
|---|---|
| -6 to -4 | TBI 200 cGy BID |
| -3 to -2 | Cyclophosphamide 60 mg/kg/dose IV daily |
| -3 to -2 | Mesna 60 mg/kg/dose IV daily (continuous infusion) |
| -1 | Rest (an additional day may be added) |
| 0 | HSCT Infusion |

**Stem Cell Infusion**

Transplant Day 0: $\geq$4 x 10$^6$/kg CD34+ stem cells by IV infusion

**NK Cell Infusion**

Post-transplant Day 21 (± 3 days): (1 x 10$^5$, 1 x 10$^6$ or 1 x 10$^7$) NK cells/kg by IV infusion. Followed by a second NK cell infusion of the same cell dose, on Day 49 ± 7 days.

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# 1 INTRODUCTION

## 1.1 OBJECTIVES

### 1.1.1 Primary

1. Assess the feasibility and toxicity of infusing escalating doses of unrelated or related donor-derived activated NK cell donor lymphocyte infusions (NK-DLI) on days 21 ± 3 days, and 49 ± 7 days following allogeneic human leukocyte antigen (HLA)-matched T cell depleted (TCD) peripheral blood stem cell transplantation (PBSCT) in patients with high risk hematologic malignancies (leukemias and lymphomas).
2. Determine if TCD allogeneic HLA-matched PBSCT + NK-DLI results in rapid, sustained donor lymphoid engraftment (>95% at day 100) and acceptable rates of acute graft-versus-host-disease (aGVHD) (<25% incidence of grade 3 or grade 4).

### 1.1.2 Secondary

1. Investigate the incidence of cGVHD in patients receiving related or unrelated donor HLA-matched allogeneic PBSCT followed by NK-DLI.
2. Investigate the incidence of viral infection and/or reactivation in patients receiving HLA-matched allogeneic PBSCT followed by NK-DLI.
3. Compare disease-free (DFS) and overall survival (OS) of patients treated on this protocol with our previous experience using T cell-replete matched sibling PBSCT in similar patient populations.
4. Correlate post-transplant NK cell, B cells and T cells numbers with select immunologic parameters including IL-7 and IL-15 levels.
5. Retrospectively investigate the impact of KIR expression and KIR reactivity on progression free survival in patients enrolled on this study.

## 1.2 BACKGROUND AND RATIONALE

### 1.2.1 Allogeneic Stem Cell Transplant for Pediatric Solid Tumors

Advances in multimodal therapies has resulted in an overall survival rate of 75% for all children diagnosed with cancer; the greatest success has been seen in patients with ALL, the most commonly diagnosed pediatric cancer. However, the management of pediatric patients with metastatic and recurrent solid tumors remains a considerable challenge. Depending upon the histology, 25-50% of children with newly diagnosed solid tumors have metastatic disease at presentation and long-term survival rates using standard therapies for these patients vary from 5-35%. Furthermore, patients who present with non-metastatic disease but experience poor responses to frontline therapy or early recurrence (within 24 months of new diagnosis) have dismal long-term outcomes. In addition, patients with desmoplastic small round cell tumor appear to have uniquely poor outcomes, even when they present without evidence for disseminated disease. Currently, salavage regimens for pediatric solid tumors have response rates in the range of 20-30% and rarely lead to durable responses(1). There are several targeted agents (eg. anti-IGFR-1, anti-VEGF) in phase I and II development that hold promise in pediatric solid tumors, however these agents are active in limited diseases and appear not to lead to durable responses or remissions.

Despite these poor overall outcomes for patients with primary metastatic, early recurrent or poorly responsive solid tumors, standard therapeutic multimodality approaches can mediate impressive antitumor effects. We have therefore sought to develop immune based therapies that can be incorporated into the standard therapeutic armamentarium for patients with ultra high-risk pediatric sarcomas. Among the approaches under study include the use of allogeneic stem cell transplantation. The rationale for undertaking this approach for ultra high-risk pediatric solid tumors is based upon several factors. First, immune reactivity occurring in the setting of allogeneic stem cell transplantation mediates impressive effects in hematologic malignancies, with graft-versus-leukemia providing substantial antileukemic effects in CML, modest effects in AML and lesser but present effects in ALL. Second, preclinical studies have demonstrated that the allogeneic environment may be particularly ripe for administering immune based therapies including tumors vaccines, adoptive T cell therapy and NK cell therapy. Third, an allogeneic GVT effect has been seen in both myeloablative and reduced intensity transplantation (RIT) for a variety of solid tumors including breast (2, 3), colon (4), renal (4, 5), ovarian, and pancreatic cancers (6). Fourth, improvements in HLA typing and supportive care, and the introduction of reduced-intensity conditioning regimens has made allogeneic HSCT safer and may improve outcomes in this heavily pre-treated population(7), thus providing new options for incorporating this therapy into a complex multimodality regimen for solid tumors. Indeed, reduced-intensity alloHSCT has been proven to be a well-tolerated, feasible approach of therapy for a variety of pediatric tumors; neuroblastoma (8 , 9-11) and pediatric sarcoma (12, 13)[POB 02-C-0259 unpublished experience], with the results comparable in terms of tumor response and disease control with those observed using conventional approaches beyond second-line therapies.

Fifth, while benefit from allogeneic HSCT for pediatric solid tumors is not proven, several groups undertaking this therapy have had encouraging results. A number of case reports have utilized allogeneic HSCT in Ewing sarcoma and have suggested a possible GVT effect for this disease(12-16). Experience with allogeneic HSCT for rhabdomyosarcoma has been limited, with one case series reporting a lack of benefit after using matched sibling PBSCs(17), but others reporting more success using matched sibling HSCT from PBSCs and bone marrow(18, 19). Whether GVT effects occur in neuroblastoma patients following allogeneic HSCT is not well established. Early studies failed to demonstrate a survival benefit comparing autologous to allogeneic HSCT(8, 20), however, these studies were not randomized and a larger percentage of high-risk patients were in the allogeneic transplant group (20). More recent case reports have reported some benefit, including one using mismatched cord-blood(21) as well as haploidentical bone marrow(10) and matched PBSCs(11). One case series using a reduced-intensity prep followed by haploidentical PBSCs showed engraftment in 6/6 patients with neuroblastoma, Ewing sarcoma or rhabdomyosarcoma, with NK cell engraftment completed within 4 weeks post-transplant. The 2 patients that were still alive had refractory neuroblastoma(22).

The POB has completed a NCI POB 02-C-0259, A *Pilot Study of Allogeneic/Syngeneic Blood Stem Cell Transplantation in Patients with High-Risk and Recurrent Pediatric Sarcomas*, the results of which serve as the basis for the backbone of this study. The EPOCH regimen has been studied extensively at the NCI and consists of etoposide, vincristine and doxorubicin administered as a continuous infusion for four days, cyclophosphamide administered as a bolus on day 5, and prednisone given orally for five days. The agents contained in this regimen are known to have activity against pediatric sarcomas and other solid tumors. Fludarabine was added the EPOCH regimen to enhance the degree of immune depletion. In this study, as well as several studies in

adults in the ETIB, the EPOCH-fludarabine pre-transplant immune depleting regimen was well tolerated, achieving effective CD4 depletion and patients who received allogeneic transplant following this regimen experienced rapid engraftment (2, 3, 23-25). Cyclophosphamide and melphalan are classical bifunctional alkylators, with a steep dose-response curve, are non-cross-resistant, differing nonhematologic toxicities, and are potentially synergistic. The most common toxicity of this combination is myelotoxicity and mucositis (26, 27). The use of combination of melphalan, cyclophosphamide and fludarabine has been successfully employed in several studies (28, 29) including 02-C-259 conducted in the POB.

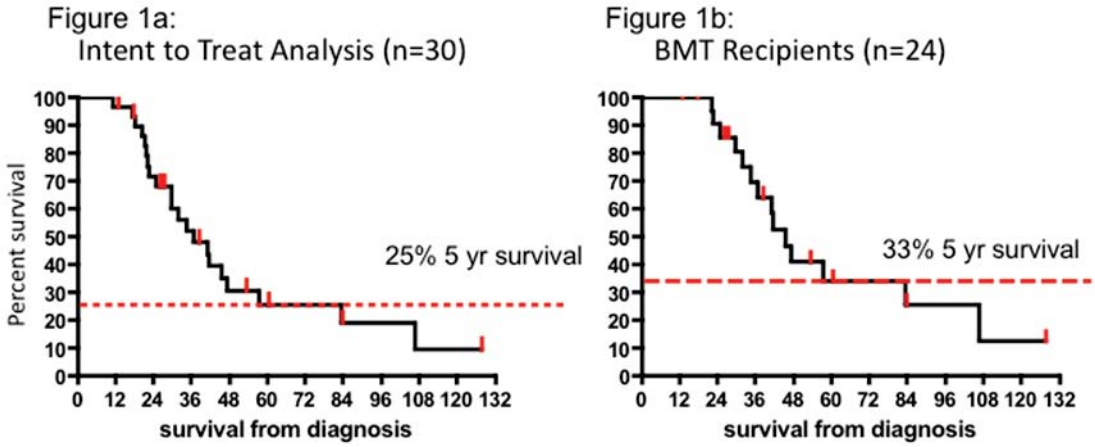
To date, a total of 30 donors and 30 recipients have been enrolled on 02-C-0259. Of these, 23 patients have undergone alloPBSCT while 7 patients were removed from study prior to transplant due to progressive disease. To achieve antitumor effects and recipient immune depletion, patients received 1-3 cycles of a fludarabine-based induction regimen in combination with EPOCH followed by a preparative regimen of cyclophosphamide (1,200 mg/m$^2$/day) and fludarabine (30 mg/m$^2$/day) for 4 days and one dose of melphalan (100 mg/m$^2$). Unmodified peripheral blood stem cell (PBSC) grafts were collected from 5-6/6 HLA-matched first-degree relatives. All donors were mobilized with 10 µg/kg/day of filgrastim for 5 days, and apheresis was performed to collect a minimum of 4 x 10$^6$/kg CD34+ PBSCs. Successful collection was achieved in all donors with a single apheresis, except for one due to a technical problem with apheresis and not from poor mobilization. Of the 24 patients transplanted, there has been no transplant related mortality (death within 100 days due to transplant complications), peri-transplant morbidity has been modest with short hospital stays (25 day median), hematopoietic recovery has been rapid (day + 11 is median day to ANC >500 and day +12 is median day to platelet > 20K), and full donor engraftment has occurred in 100% of patients by day + 28. Donor engraft was independent of CD4 count at entry or prior to transplant. Patients with pre-transplant counts as high as 490/mm3 engrafted rapidly and there was no mixed chimerism or graft rejection encountered.

In the initial cohort (cohort 1, n=12), GVHD prophylaxis consisted of cyclosporine as a single agent. Although high-grade (grade III-IV) aGVHD was not observed, we observed a high incidence of low-grade aGVHD and universal occurrence of cGVHD (12/12 evaluable patients). The high incidence of GVHD precluded donor lymphocyte infusion (DLI) and required systemic immunosuppression in many patients, therefore the GVHD prophylaxis was modified from cyclosporine alone in cohort 1 to tacrolimus and sirolimus in cohort 2. The second cohort (n = 11) has shown similarly low peri-transplant morbidity and full donor engraftment in all patients. Further, the rates of aGVHD are significantly reduced, with 3/11 patients developing grade 1, one patient with grade 3, and one patient with grade 4 aGVHD. The incidence of cGVHD in the second cohort appears to show improvement with the new immunosuppression therapy with 3/10 evaluable patients having extensive cGVHD and 2/10 with limited cGVHD. Despite the decrease in overall GVHD rates and severity, not all patients tolerated the prophylactic regimen consisting of tacrolimus and sirolimus and cGVHD occurred almost universally in those who did not receive the complete course of immunosuppression with both agents and no patients received DLI.

Thus, the regimen used in cohort 2 of 02-C-0259 accomplished good engraftment, acceptable toxicity and acceptable rates of aGVHD and cGVHD. When evaluating for disease control during EPOCH-F therapy, 2 patients had partial responses (PR), 11 patients had stable disease (SD), 5 remained with no evidence of disease (NED), and 12 patients had progressive disease (7 of these patients did no go onto transplant. At the day 100 evaluation 11 patients had progressive disease,

5 patients had stable disease, 5 patients remained NED and 2 patients previously with disease became NED. Graft versus tumor effect was demonstrated in one patient with a skull-based lesion that emerged and subsequently regressed after transplant. In addition, several patients who went into tranplant with chemotherapy refractory disease were then chemosensitive following transplant, including 5 patients that became NED with post-transplant EPOCH therapy. Furthermore, median survival of patients on 02-C-0259 was higher than that for a historical control group identified *a priori* with similarly high-risk disease (median survival from diagnosis of historical controls 18 mos. vs. 36 month of all patients enrolled on 02-C-0259 vs. 45 months for patients receiving BMT on 02-C-0259). Despite these promising results, disease recurrence remains a substantial problem. **Figure 1**a shows the survival of all patients enrolled on 02-C-0259 from the time of enrollment using an intent-to-treat approach. **Figure 1**b shows survival of all patients receiving transplant. Five year overall survival was 25% using the intent-to-treat analysis and 33% in BMT recipients, which is comparable to historical controls for this ultra high-risk patient population. Moreover, all but one patient experienced disease recurrence, and many require chronic therapy with chemotherapy to sustain disease control. Thus, the antitumor effects of this allogeneic stem cell transplant regimen is not adequate to cure these ultra high risk patients.

Figure 1




Based upon these results, our goal for the current study is to use a similar platform, but to attempt to improve disease free survival by augmenting graft-versus-tumor activity. Graft-versus-tumor effects can be T cell mediated or NK cell mediated. Because of our concerns regarding the high rate of GVHD and the requirement for immunosuppression in order to treat this disorder, the current study will seek to completely eliminate GVHD using T cell depletion and focus on augmentation of NK reactivity. Evidence from preclinical studies concluded that NK cells do not initiate GVHD and therefore it is predicted that NK cell infusions will not induce GVHD. Furthermore, activated NK cells can now be readily generated and they show potent killing activity of pediatric solid tumors and ALL blasts. On this basis, we will seek to build upon our previous platform by maintaining the previous pre-transplant and peri-transplant preparative regimen, but incorporate T cell depletion and escalating NK cell infusions in an attempt to enhance graft-versus-tumor effects.

### 1.2.2 Pediatric Leukemias and Lymphoma

As a group, childhood leukemias represent the most common pediatric malignancy, accounting for approximately 32% of cancer in children younger than 15 years and 25% under 20 years of age. ALL is by far the most common, comprising approximately 23% of childhood cancer with an annual rate of 30 to 40 new cases per million U.S. children. AML accounts for approximately 4% of pediatric cancer diagnoses and 20% of childhood leukemia with an annual rate in the U.S. of 8 per million. Ph+ CML is rare and accounts for approximately 1% of all pediatric cancer, although it comprises 10% of leukemia in older adolescents. Although the majority of pediatric patients with leukemia are cured, leukemia remains the second most frequent cause of death from cancer in children. Similarly, cure rates are excellent for both Hodgkin's disease and non-Hodgkin's lymphomas (NHL) in children, however outcomes are poor for those with refractory disease and for those who relapse after standard salvage therapies such as autologous bone marrow transplant (ABMT). Conventional alloHSCT appears to offer benefit over ABMT and can be used for salvage in those settings.

There have been no large prospective randomized controlled clinical trials to definitively evaluate the role of alloHSCT versus chemotherapy in the management of childhood ALL. This is due in part to the success of standard front-line chemotherapy and the inherent variability in stem cell donor availability. Nonetheless, multiple comparative studies indicate that relapse rates are lower after alloHSCT in comparison to chemotherapy, and alloHSCT is standard of care therapy for patients in CR#2 or for select very high-risk patients in CR#1(30). Our branch has experience delivering a reduced intensity alloHSCT to leukemia and lymphoma patients enrolled on protocol 01-C0125 using a reduced intensity platform similar to that described above for protocol 02-C-0259. Lymphoma patients received cycles of EPOCH-F, followed by a non-myeloablative PBSCT with a preparative regimen consisting of cytoxan and fludarabine. Leukemia patients received 3 cycles of FLAG chemotherapy, followed by the same preparative regimen consisting of cytoxan and fludarabine. A total of 15 recipients have undergone alloHSCT on this protocol. Immune depletion with pre-transplant therapy was achieved in all subjects and all patients tolerated induction chemotherapy cycles with anticipated side effects. The most common side effects included nausea, vomiting, abdominal pain, elevated transaminases, neutropenia, thrombocytopenia, anemia, neutropenic fever, infection, electrolyte abnormalities, and hypertension. Hematopoietic recovery was rapid and complete for all subjects with median times to ANC >500/µL of 10 days (range, 7-13) and platelet count >20,000/µL of 11 days (range, 8-16, 1 not evaluable). There has been rapid lymphoid recovery post-transplant with evidence for early recovery of thymic derived T-cells. CD4, CD8, and NK cell counts increased to levels above baseline 1 month after transplant. Similar to the solid tumor study, donor engraft kinetics were independent of CD4 count at entry or prior to transplant. All patients engrafted rapidly and there was no mixed chimerism or graft rejection encountered. Thirteen of 15 recipients developed aGVHD and 12 of 13 with > 100 days of follow-up have developed cGVHD. GVHD has responded to therapy in all patients. Although there have been no deaths directly attributable to GVHD on this trial, this protocol was modified to use bone marrow rather than PBSCs to reduce cGVHD incidence and severity.

Eight of 15 patients (50%) remain alive (median follow-up 34 mo; range 3-81 mo), six (40%) have died of relapsed leukemia or lymphoma, and 1 died of toxicity. Six of 15 patients (40%) remain in continuous CR (3-60 mo) and 2 (14%) are alive with disease. Of the patients with relapse, only 1 patient relapsed prior to day 100 (day 95). A graft-vs.-leukemia effect was clinically demonstrated

in 2 patients. In addition, anti-tumor alloreactivity was suggested by apparent enhanced chemotherapy responses in 3 patients with Hodgkin's. In summary, this non-myeloablative platform can reproducibly induce full donor engraftment with acceptable treatment related mortality for patients with high risk hematologic malignancy. At the same time, disease recurrence remains the major cause for treatment failure therefore new approaches are needed to enhance antitumor effects of non-myeloablative HSCT for hematologic malignancies.

### 1.2.3 Current Standard of Care for Eligible Patients

#### 1.2.3.1 Ewing Sarcoma (**This study is no longer enrolling patients with solid tumors as of Amendment K [see section 1.4.1.3]**)

Prognosis of patients with metastatic disease is extremely poor with 6-year event-free survival (EFS) of approximately 28% and overall survival (OS) of approximately 30%. Patients with bone/bone marrow metastases have a 4-year EFS of approximately 28% and patients with combined lung and bone/bone marrow metastases have a 4-year EFS of approximately 14%. The overall prognosis for patients with recurrent Ewing sarcoma is equally poor with 5-year survival rates following recurrence of 10% to 15%. For recurrent patients, time to recurrence is the most significant prognostic factor. Patients who recurred greater than 2 years from initial diagnosis (approximately 1 year after completion of therapy) have a 5-year survival of 30% versus 7% for patients who recurred within 2 years (31-33).

Standard treatment for patients with metastatic Ewing tumor of bone or bone marrow uses alternating vincristine, doxorubicin, cyclophosphamide, and ifosfamide/ etoposide combined with local control (surgery and/or radation) with an overall cure rate of approximately 20%. More intensive therapies, incorporating high-dose chemotherapy with autologous stem cell support, have not shown improvement in EFS (31).

There are no standardized treatments for patients with recurrent Ewing sarcoma. Combinations of chemotherapy such as gemcitabine and taxotere, cyclophosphamide, topotecan or irinotecan, and temozolomide are active in recurrent Ewing sarcoma family of tumors and can be considered for these patients, as well as phase I therapy or palliative care (34).

#### 1.2.3.2 Soft Tissue Sarcomas – Rhabdomyosarcoma (RMS), Desmoplastic Small Round Cell Tumor (DSRCT) (**This study is no longer enrolling patients with solid tumors as of Amendment K [see section 1.4.1.3]**)

Despite very aggressive therapy, the prognosis for children with metastatic soft tissue sarcomas is poor, 37-50% for RMS (35) and 5 year survival rates for DSRCT range from 15% for all patients and approach 0% for unresectable patients (36). Standard treatment includes combined treatment with chemotherapy, radiation therapy, and surgical resection of pulmonary metastases (34, 35).

There is no standardized care for patients with progressive or recurrent DSRCT or RMS. Additional treatment depends on multiple factors, including the sites of recurrence, previous treatment, and individual patient concerns. Treatment for local or regional recurrence may include wide local excision or aggressive surgical removal of tumor, radiation therapy should be considered for patients who have not already received it in the area of recurrence. Previously unused, active, single agents or combinations of drugs may also enhance the likelihood of disease control and phase I and phase II trials should be considered (34).

1.2.3.3 Neuroblastoma (**This study is no longer enrolling patients with solid tumors as of Amendment K [see section 1.4.1.3])**

Neuroblastoma is the most common extracranial solid tumor in children, accounting for 6-8% of all childhood cancers and over 15% of pediatric cancer deaths (37). For children with high-risk neuroblastoma, long-term survival with current treatments is approximately 30%. Children with aggressively treated, high-risk neuroblastoma may develop late recurrences, some more than 5 years after completion of therapy (38, 39). Neuroblastoma risk groups have been defined by the Children's Oncology Group (COG) as follows:

**Table 1. Children's Oncology Group Neuroblastoma Low-, Intermediate-, and High-Risk Group Assignment Schema Used for COG-9641 and COG-A3961 Studies[a]**

| INSS Stage | Age | *MYCN* Status | INPC Classification | DNA Ploidy[b] | Risk Group |
|---|---|---|---|---|---|
| 1 | 0–21 y | Any | Any | Any | Low |
| 2A/2B[c] | <365 d | Any | Any | Any | Low |
| | ≥365 d–21 y | Nonamplified | Any | - | Low |
| | ≥365 d–21 y | Amplified | Favorable | - | Low |
| | ≥365 d–21 y | Amplified | Unfavorable | - | High |
| 3[d] | <365 d | Nonamplified | Any | Any | Intermediate |
| | <365 d | Amplified | Any | Any | High |
| | ≥365 d–21 y | Nonamplified | Favorable | - | Intermediate |
| | ≥365 d–21 y | Nonamplified | Unfavorable | - | High |
| | ≥365 d–21 y | Amplified | Any | - | High |
| 4[d] | <548 d [13-15] | Nonamplified | Any | Any | Intermediate |
| | < 365 d | Amplified | Any | Any | High |
| | ≥548 d–21 y | Any | Any | - | High |
| 4S[e] | <365 d | Nonamplified | Favorable | >1 | Low |
| | <365 d | Nonamplified | Any | =1 | Intermediate |
| | <365 d | Nonamplified | Unfavorable | Any | Intermediate |
| | <365 d | Amplified | Any | Any | High |

*[a]The COG-9641 and COG-A3961 trials established the current standard of care for neuroblastoma patients in terms of risk group assignment and treatment strategies.*

*[b]DNA Ploidy: DNA Index (DI) > 1 is favorable, = 1 is unfavorable; hypodiploid tumors (with DI < 1) will be treated as a tumor with a DI > 1 (DI < 1 [hypodiploid] to be considered favorable ploidy).*

*[c]INSS stage 2A/2B symptomatic patients with spinal cord compression, neurologic deficits, or other symptoms should be treated with immediate chemotherapy for four cycles.*

*[d]INSS stage 3 or stage 4 patients with clinical symptoms as listed above should receive immediate chemotherapy.*

*[e]INSS stage 4S infants with favorable biology and clinical symptoms should be treated with immediate chemotherapy until asymptomatic (2–4 cycles). Clinical symptoms include: respiratory distress with or without hepatomegaly or cord compression and neurologic deficit or inferior vena cava compression and renal ischemia; or genitourinary obstruction; or gastrointestinal obstruction and vomiting; or coagulopathy with significant clinical hemorrhage unresponsiveeplacement therapy.*

Patients classified as high risk receive treatment with an aggressive regimen of induction chemotherapy consisting of high doses of the following agents: cyclophosphamide, ifosfamide, cisplatin, carboplatin, vincristine, doxorubicin, etoposide, and topotecan. After a response to chemotherapy, resection of the primary tumor should be attempted, followed by myeloablative chemotherapy and stem cell rescue. In addition, radiation to the primary tumor site should be undertaken whether or not a complete excision was obtained. After recovery, patients are treated

with oral 13-cis-retinoic acid for an additional 6 months. For high risk patients in remission following HSCT, the chimeric anti-GD2 antibody ch14.18 combined with granulocyte-macrophage colony stimulating factor (GMCSF), interleukin-2 (IL 2), in conjunction with retinoic acid improves has been found to improve EFS and is now standard of care (34, 40).

For patients initially classified as high risk that relapse or have progressive disease the prognosis is exceedingly poor and phase I or phase II clinical trials are appropriate and should be considered (41).

#### 1.2.3.4 Acute Myelogenous Leukemia (AML)

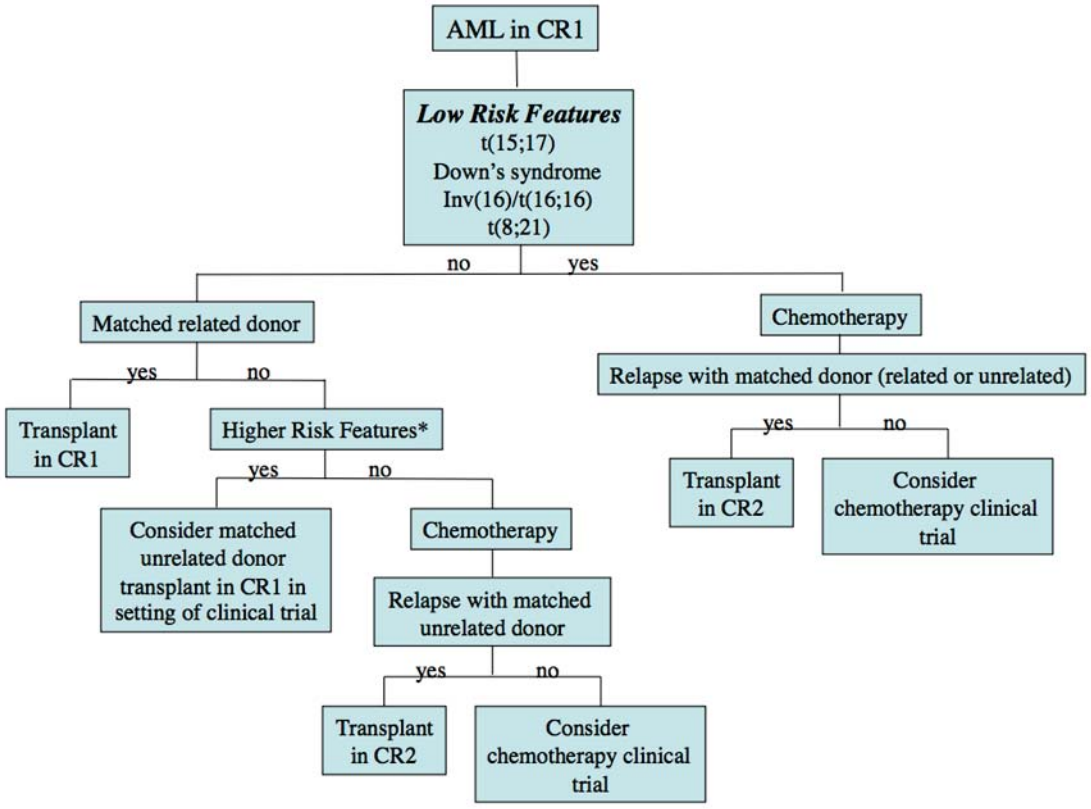
Non-Transplant Therapy: In most cases, AML treatment consists of intensive induction, consolidation, and CNS-directed chemotherapy. Approximately 75 to 90% of children with AML will achieve a CR and increasing the treatment intensity of induction improves DFS rates (42-44). Post-remission consolidation chemotherapy is essential and can be delivered in standard doses or as high-dose therapy with autologous stem cell rescue with similar DFS rates (45-48). Despite treatment intensification the outcome is guarded for most children with AML, and only about 50% are cured with chemotherapy alone (49). Individuals with acute promyelocytic leukemia (FAB M3) have a better prognosis, with 80% DFS rates observed when all-trans-retinoic acid is added during induction and a maintenance phase (50-53). Young children with trisomy 21 who develop AML also have excellent outcomes and require less intensive therapy (54-56).

Transplantation: Given the relative poor outcome for pediatric patients with AML, allogeneic HSCT has commonly been used as consolidation in CR1. There have been multiple "genetic randomization" studies of matched related allogeneic HSCT in which individuals who have matched sibling donors are assigned to transplantation. Allogeneic HSCT confers a lower risk of relapse and improves DFS in comparison to chemotherapy with or without autologous rescue (42-44, 54, 57-62). However, clinical benefits can be offset by transplant-related morbidity and mortality, which may eliminate any overall survival advantage in low risk groups (43, 63-66). Consequently, there is some debate as to whether allogeneic HSCT should be employed in CR1 or CR2 for AML in childhood (66-68). The ASBMT has published consensus guidelines for the use of HSCT in pediatric AML (57) and a suggested approach is presented in **Figure 2**.

In the U.S., matched related sibling donor HSCT is the most common consolidation therapy employed for children with AML in CR1 outside of specific low risk groups. This approach is based largely on clinical trials conducted by the Pediatric Oncology Group (POG) and the Children's Cancer Group (CCG) (69, 70). Both groups reported superior outcomes for high and intermediate risk patients treated with HSCT in CR1. The 5-year overall survival for patients transplanted with a matched sibling donor in CR1 ranges from 52% to 72% (42-44, 46, 57, 62, 69, 70).

Long-term DFS can be achieved in approximately 30% of children with AML who are transplanted in CR2 with either matched unrelated or mismatched related donors (62). Consequently, HSCT is sometimes reserved for management of patients who relapse after chemotherapy, especially for low risk groups (68) or those without sibling donors.

**Figure 2**




1.2.3.5 Acute lymphoblastic leukemia (ALL)

Clinical and biologic features are used to subtype, risk-stratify and assign therapy at diagnosis. Initial risk group assignment is made based on age, peripheral white blood cell count (WBC), central nervous system (CNS) involvement, and phenotype (71). Phenotypic classification is determined by flow cytometry of lineage-associated cell surface markers. The majority of ALLs are of precursor B-cell (pre-B) phenotype (CD10, CD19, HLA-DR, TDT +), 10 to 20% are T-cell (CD2, CD3, CD5, and/or CD7 +), and <5% are mature B-cell or Burkitt-type (CD20, surface-IgM+).

Cytogenetic studies are subsequently used to further define the risk of relapse. The t(12;21) translocation, the most frequent recurrent chromosomal translocation associated with childhood ALL, is identified in approximately 25% of cases and this is associated with a favorable prognosis (72-75). Gene rearrangements of the mixed-lineage leukemia (MLL) gene located at 11q23 is the most common cytogenetic finding in infants with ALL, which has an extremely poor prognosis (76-79). The so called Philadelphia chromosome (Ph+), which results from a translocation between chromosomes 9 and 22, t(9;22), also confers adverse risk (80). The t(1;19) translocation is also associated with an increased risk of relapse, but this can be offset by therapy intensification (81,

82). Hyperdiploidy, which most often includes trisomies of chromosomes 4, 7, and/or 10, carries a favorable prognosis (83-86). Hypodiploid cases are at higher risk of relapse (87-90).

The prognosis after relapsed ALL depends on the duration of the first remission (CR1) and the site of relapse (91-94). Outcome after short CR1 duration (<12-18 months) is very poor, as is the prognosis for individuals who are unable to achieve a second remission. Those with isolated extramedullary relapse fair better than those with marrow relapse (95, 96).

There have been no large prospective controlled clinical trials to evaluate the relative efficacy of allogeneic HSCT in comparison to chemotherapy for childhood ALL. However, multiple comparative studies suggest that relapse rates are lower after HSCT (30). Some of the benefits in regard to relapse-free survival are offset by transplant-associated morbidity and mortality (97). Consequently, HSCT is usually reserved for the management of relapse and it is rarely employed for children in CR1 except for those with extremely high-risk features (Figure 3). For those with HLA-matched sibling donors, allogeneic HSCT in second remission is considered standard. Unrelated donor HSCT is usually reserved for those at high risk of relapse with chemotherapy. Importantly, the approach in individual cases will vary based on risk/benefit analysis, donor options, and access to transplantation. The American Society for Blood and Marrow Transplantation (ASBMT) has published consensus guidelines for the use of HSCT in childhood ALL (30) and a suggested algorithms for HSCT in pediatric ALL based on these recommendations are presented in **Figure 3** and **Figure 4**.

**Figure 3**

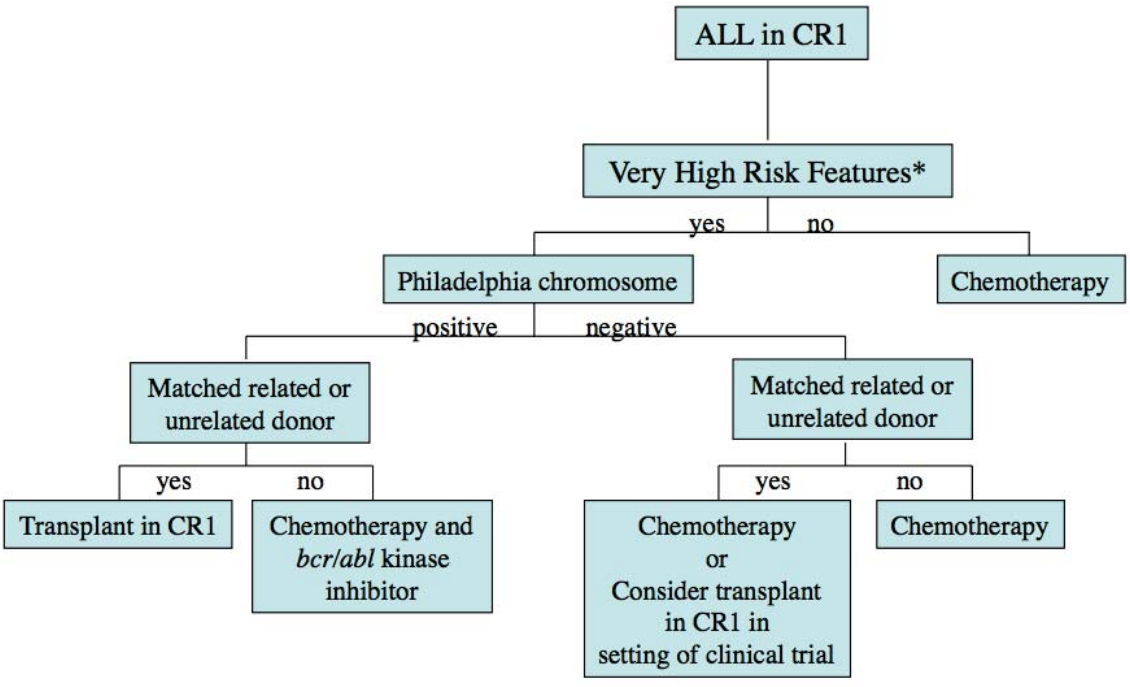
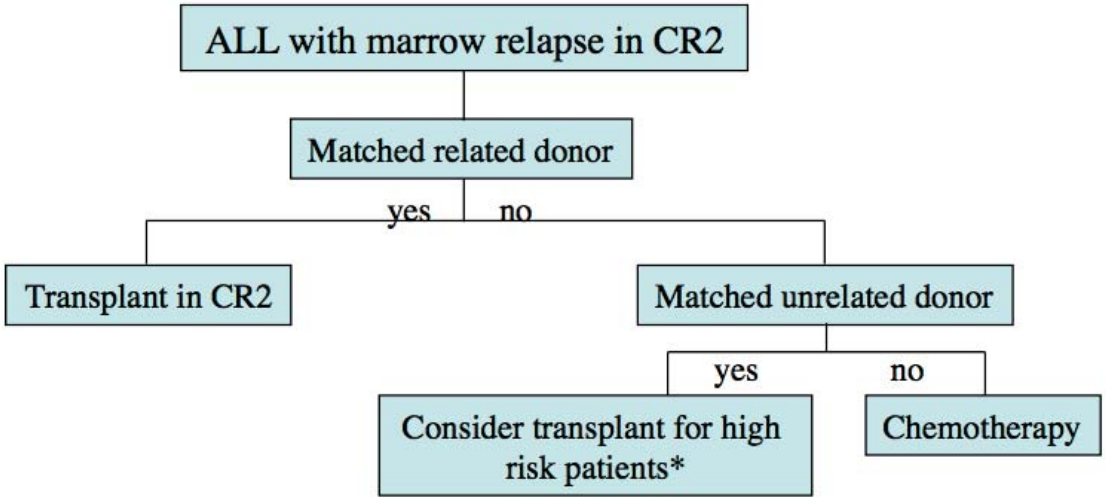


**Figure 4**




A retrospective matched cohort analysis performed by the Children's Oncology Group (COG) and International Bone Marrow Transplant Registry (IBMTR) compared matched related HSCT to chemotherapy for children with ALL in CR2. Leukemia-free survival and relapse rates were better after HSCT in all patient groups regardless of the CR1 duration (97).

HSCT in first remission has no proven benefits for patients defined as high-risk by WBC count, gender, and age. However, transplantation is commonly considered for those at very high risk of relapse with standard therapy (e.g., hypodiploidy, induction failure) (**Figure 3**). Although historically HSCT has been considered for children with Ph+ ALL (80, 98), the addition of the tyrosine kinase inhibitor imatinib mesylate to chemotherapy appears to have improved non-transplant outcome (99). Importantly, the use of imatinib in the patient population allows more patients to achieve a CR and improves transplant outcomes. Standard of care remains transplant in CR1 for these very high risk patients (100).

1.2.3.6 Chronic Myelogenous Leukemia (CML)

CML is characterized by the presence of the Philadelphia chromosome and the associated translocation product bcr/abl. CML has three defined clinical phases: chronic, accelerated, and blast crisis, with most patients presenting with chronic phase. Response to treatment and survival correlate with phase of disease. Blast crisis is clinically indistinguishable from acute leukemia and treatment responses are short lived.

Non-Transplant Therapy: The kinase activity of the bcr/abl fusion protein is inhibited by imatinib mesylate (Gleevec) and related kinase inhibitors, and these agents have transformed the approach to treatment with CML (101). Imatinib induces complete remissions in most patients with chronic phase CML, although continuous treatment appears to be required and resistance may develop (102, 103). Thus, there is as of yet no evidence that this new class of kinase inhibitors will be curative and it is unclear that TKIs be recommended as a replacement for allogeneic HSCT in children who have an HLA-matched donor (104, 105). A number of criteria have been proposed for deciding when to proceed from kinase inhibitor therapy to HSCT, including loss of therapeutic

response or failure to achieve a complete hematologic response by 3 months or a substantial cytogenetic response by 3 to 6 months of treatment (106).

Allogeneic HSCT is the only proven cure for CML and donor availability should be considered soon after diagnosis for all children with this disorder. Post-transplant DFS rates are inversely related to age and exceed 80% for young children with matched sibling donors in first chronic phase. Results are best when HSCT is performed in first chronic phase and with a shorter diagnosis-to-transplant interval. Success is substantially diminished for the accelerated phase or blast crisis and attempts should be made to induce a second chronic phase prior to transplant (107, 108). In general, pediatric patients have relatively low risk of transplant-related mortality and results are similar with matched unrelated and related donors. Thus, unrelated donor HSCT is usually recommended for those who lack sibling donors (101, 104, 106, 108-110).

1.2.3.7 Juvenile Myelocytic Leukemia (JMML)

The myelodysplastic syndromes represent a heterogeneous group of disorders characterized by ineffective hematopoiesis, impaired maturation of myeloid progenitors, cytopenias, dysplastic changes, and a propensity for the development of AML (111, 112). The major diagnostic groups within MDS encountered in pediatric patients include JMML, myeloid leukemia of Down syndrome, and MDS occurring de novo and secondary to previous therapy or pre-existing disorders (113-115). In general, pediatric MDS carries a poor prognosis and clinical variables have little practical utility in guiding therapy (116-119).

JMML is resistant to therapy. Although chemotherapy may reduce disease burden, responses are usually short lived and the disease rapidly progresses with a median survival of approximately 1 year (120). The European Working Group of MDS (EWOG-MDS) in Childhood reported a retrospective analysis of 110 cases of JMML. The probability of survival at 10 years was 6% for the non-transplant group vs. 39% after transplantation (117).

HSCT is considered the only curative treatment for childhood MDS and JMML. Given the low response rates to non-transplant therapies, and because failure rates after HSCT appear lower when HSCT is performed soon after diagnosis, strong consideration should be given for early transplantation, especially when a matched sibling donor is available. DFS rates of 50 to 64% are reported with HSCT. Individuals with JMML who develop GVHD have a lower incidence of relapse (111, 121).

Outcome may be improved for individuals transplanted with lower blast percentage and induction chemotherapy is commonly employed for patients with elevated bone marrow blasts to induce a CR prior to HSCT (119, 122). However, definitive recommendations cannot be made given the paucity of data. Given the poor prognosis without transplant and the favorable results of matched unrelated donor HSCT in pediatrics, transplantation is usually recommended for children with JMML and MDS without regard to the donor type (112, 123-126).

1.2.3.8 Hodgkin's and Non-Hodgkin's Lymphoma

Hodgkin's Lymphoma:

Treatment failure in children and adolescents with Hodgkin lymphoma is typically divided into three groups: 1.) Primary progressive disease; 2.) Relapse limited to the site(s) of initial involvement (in patients treated with chemotherapy alone); 3.) other relapse. Salvage rates for first recurrence range from 10 year OS rates of 55-90% (127-133).

Initiatial salvage therapy for Hodgkin's Lymphoma for patients who did not receive prior radiotherapy includes further chemotherapy and low-dose involved-field radiation. For the remaining patients, treatment of relapse/progression includes an induction chemotherapy, followed by high-dose chemotherapy with hematopoietic stem cell transplant (HSCT). Studies show better outcomes following autologous versus allogeneic stem cells transplant(134). Following autologous HSCT, the projected survival rate is 45% to 70% and progression-free survival is 30% to 65% (135, 136). For patients who fail following autologous HSCT or for patients who cannot mobilize sufficient numbers of autologous stem cells, allogeneic HSCT has been used with encouraging results (134, 137-139).

Recurrent Non-Hodgkin's Lymphoma:

For recurrent or refractory B-lineage non-Hodgkin lymphoma (NHL) or lymphoblastic lymphoma, survival is generally 10% to 20% and approximately 60% for recurrent or refractory anaplastic large cell lymphoma (140). There is no current standard treatment option for patients with recurrent or progressive disease. Disease control can be achieved in 40% of NHL patients using aggressive salvage regimens such as dexamethasone, etoposide, cisplatin, cytarabine, and asparaginase, maintenance chemotherapy, and transplantation (141). Radiation therapy may have a role in treating patients who have not had a complete response to therapy. Rituximab with or without cytotoxic chemotherapy has also shown activity in the treatment of B-cell lymphoma patients(142). If remission can be achieved, high-dose therapy and stem cell transplantation are usually pursued. All patients with primary refractory or relapsed NHL should be considered for clinical trials (34).

### 1.2.4 Matched Unrelated Donor Transplant in Pediatrics

Only 25 to 30% of patients have a sibling donor who is fully matched at the class I and II major histocompatibility complex (MHC) loci, which encode for the human leukocyte antigens (HLA-A, B, C, DR, DQ, and DP). Since 2005, approximately 22 (otherwise eligible) potential candidates for the POB sarcoma transplant protocol (02-C-0259) were screened but found to be ineligible secondary to the lack of a suitable HLA-compatible sibling donor. During this same time period, 20 patients were HLA-typed and found to have a matched sibling donor. Of these, only three patients did not come to the NIH for further eligibility screening for transplant (one because of disease progression). The remaining 17 patients were evaluated and enrolled onto protocol. This limitation prompted the exploration of alternative stem cell sources for allogeneic HSCT including the use of HLA-matched unrelated donors (MUD). Enrollment of volunteer donors in national and international registries, such as the NMDP of the United States, has facilitated the identification of unrelated stem cell donors for patients without HLA-matched sibling donors. Historically, transplants from unrelated donors were complicated by higher rates of graft rejection, acute and chronic GVHD, immune dysregulation, and treatment-related mortality. However, these complications were partially balanced by lower relapse rates (143, 144) and in more recent years, complications have been reduced to the levels of matched related donor transplants (144, 145).

**Table 1) Unrelated Donor Bone Marrow Transplantation: Pediatric Series**

| Patients (n) | Probability of myeloid engraftment | aGVHD grades 2-4 | aGVHD grades 3-4 | cGVHD | Transplant Related Mortality | Reference |
|---|---|---|---|---|---|---|
| MUD (46)<br>1 MMUD (42) | 100% | 83%<br>98% | 37%<br>62% | 60%<br>69% | 24%<br>51% | Balduzzi 1995 |
| MUD (8)<br>1 MMUD (9) | 83% | 63% | 37% | 54% | N/A | Davies BJH 1997[γ] |
| MUD (30)<br>1 MMUD (20) | 93% | 49%<br>67% | 23%<br>30% | 50%<br>55% | Day 100 NRM 36% | Davies JCO 1997 |
| MUD (28)<br>MRD (37) | N/A<br>N/A | 64%<br>38% | 32%<br>14% | 57%<br>26% | 11%<br>19% | Saarinen-Pihkala 2001 |
| 0-1 MMUD (363) | 98% | 47% | 29% | 39% | Day 100 27%<br>5-year 42% | Bunin 2002 |
| MUD (56)<br>MMUD (32) | 98% | 85% | 43%<br>59% | 58% | 35% | Woolfrey 2002 |
| MUD (33)<br>1 MMUD (22)<br>2-3 MMUD(11) | 97% | 40%<br>42%<br>37% | 7%<br>9%<br>9% | 20%<br>34%<br>40% | Day 100 10%<br>Day 180 15%<br>Overall 22% | Giebel 2003[ψ] |
| MUD (23) | 100% | 48% | 14% | 46% | 48% | Talano 2006 |

N/A = not available, n = number, RD = related donor, MUD = matched unrelated donor, MMUD = mismatched unrelated donor, MM = mismatched, mMUD = molecular MUD, sMUD = serologic MUD, TRM = transplant related mortality, *high resolution typing (10 allelle), [ψ] 4 received peripheral blood stem cells, [γ] data from all patients (0-1 MM) combined, NRM = non-relapse mortality.

Importantly, pediatric patients appear to tolerate alternative donor transplants (MUD and mismatched related donors) better than adults. Studies of unrelated donor BMT in the pediatric population during the 1980's through the early 1990's report high incidences of aGVHD (37-62%) and cGVHD (50-69%) (**Table 1**)(143, 144, 146-151). More recent studies however, suggest a decreasing incidence of both aGVHD (21-29%) and cGVHD (39-46%) (152) (153). In 2002, investigators in Seattle published results on unrelated-donor marrow transplantation for children with ALL. All patients (n=88) received the same conditioning regimen with cyclophosphamide and TBI and most received CSA with MTX for GVHD prophylaxis. Fifty-six patients had an HLA-matched donor and 32 had a single antigen mismatch. Acute GVHD was seen in 43% of matched recipients and 59% of mismatched recipients. Of those patients surviving past day 80, 47% developed extensive cGVHD and limited cGVHD was observed in 11% (150).

1.2.4.1 Direct comparison of related to unrelated donor transplant:

A population-based study of 65 pediatric transplant patients from seven Nordic centers who underwent BMT for ALL from either matched sibling donors (n=37) or unrelated donors (n=28) was published in 2001. GVHD prophylaxis in the matched sibling group consisted of cyclosporine (CSA) in all and methotrexate (MTX) in 67% of patients. GVHD prophylaxis in the unrelated donor group consisted of CSA and MTX with 64% receiving anti-thymocyte globulin (ATG) and 11% also having TCD. Acute GVHD and cGVHD were significantly higher in the unrelated group compared to the related group (32% versus 14%; 57% versus 26%). However, the transplant-related mortality (TRM), event-free survival (EFS) and OS were not significantly different, since patients with sibling donors had higher relapse rate and earlier time to relapse (143).

Fifty-seven adult and pediatric hematologic malignancy patients who received MUD BMT were matched for disease and stage with 57 recipients of genotypically matched related donor BMT. All patients received CSA and MTX for GVHD prophylaxis and MUD patients also received ATG. For MUD vs related donor recipients, respectively, incidence of aGVHD grade II–IV was 19% vs 36%, grade III–IV 10% vs 18%, and cGVHD 44 % vs 51%. Non-relapse mortality was 15% vs 8% at 100 days, 28% vs 36% at 3 years. Also at 3 years, relapse was 45% vs 42% and disease-free survival was 39% vs 37%. None of these differences were significant. The three-year OS was identical at 42%. The authors concluded that MUD BMT recipients given pre-transplant ATG have similar outcomes to recipients of MRD BMT using conventional drug prophylaxis (152).

In 2006, Uzunel et al. reported on 137 patients who received reduced intensity conditioning for alloHSCT in Sweden. Outcomes of 63 patients receiving a sibling donor graft were compared to the outcomes of 74 patients receiving a MUD graft. Patients in the two groups were similar in terms of diagnosis, age, sex and conditioning regimen. The GVHD prophylaxis was also similar in the two groups, with the exception that ATG was given to all of the patients receiving an unrelated graft but to only 46% of the patients receiving stem cells from a sibling donor. Engraftment was successful in most patients with no significant difference between MUD and sibling transplants. No difference in severe aGVHD was found between the groups. However, the incidence of cGVHD was higher after sibling transplants. This was probably due to higher donor age in this group, since this was the only significant risk factor for cGVHD in multivariate analysis. The incidence of TRM was significantly higher after MUD transplantation (40%) than after sibling transplantation (16%) (P 0.01). However, because relapse and disease progression were more common after sibling transplantation, there was no significant difference in overall survival between the two groups (154).

Similarly, a group in Spain reported the results of 58 children with ALL after related (n = 31) or MUD (n = 27) HSCT. Characteristics at diagnosis and anti-leukemic treatments were similar in both groups. GVHD prophylaxis consisted of CSA alone or CSA and MTX in the related group, and the majority of unrelated recipients received CSA, MTX and 2/3 also received ATG. The 5-year event free survival probability was 43 +/- 9% for the related group and 36 +/- 9% in the MUD group (p = .25). The TRM was 16% in the related group and 37% in the MUD group (p = .016). The rate of relapse was higher in the related group 36.7% versus 18.6% in the MUD group (p = .05). GVHD associated with organ failure or infection caused most of the transplant-related deaths in both groups (153).

1.2.4.1.1 High-resolution typing:

A joint Polish and Italian study prospectively evaluated 63 children transplanted for hematologic malignancies from unrelated donors selected by high-resolution typing of both HLA class I and class II loci. They evaluated completely matched as well as 1-, 2-, and 3- antigen mismatched donors. They observed low incidences of severe aGVHD (7-9%), cGVHD (20-40%) and graft failure, with rates comparable to those seen in children transplanted from HLA-identical siblings (155).

When comparing unrelated to related stem cell transplants, a group in France analyzed the outcome of 89 patients (median age 17 years, range 0-52) with acquired severe aplastic anemia (SAA) undergoing HSCT from an unrelated donor between 1989 and 2004 reported to the French Registry by 25 centers. They found that patients transplanted during two successive time-periods (1989-1998 and 1999-2004) had different 5-year survival probabilities of 29% versus 50%, respectively ($p<0.01$). The main difference between the two cohorts was identified as the degree in HLA matching between donors and recipients at the allelic level for the ten HLA-A, -B, -C, -DRB1 and -DQB1 antigens, which was more frequent in 1999-2004 than in the former period ($p=0.0004$). In multivariate analysis, the only two factors affecting survival were HLA allelic matching ($p<0.01$) and younger age of recipient ($p<0.0001$). The group concluded that survival after unrelated HSCT for SAA has improved significantly over the past 15 years, mainly due to better HLA matching and that results for young patients who are fully HLA-matched at the allelic level with their donor are comparable to those observed after HSCT from a related donor (156). Additionally, in a recent review of 3,857 transplants reported to the NMDP, the authors found that high-resolution typing was associated with higher rates of overall survival (157).

**Figure 5**

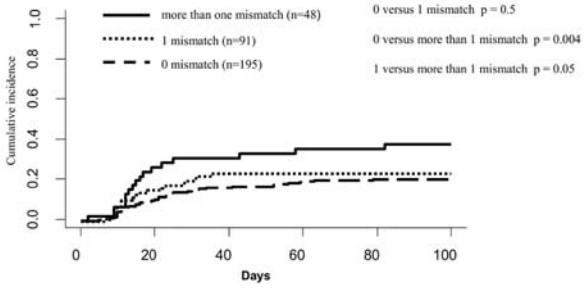



**Figure 1.** Effect of number of HLA-A, -B, -C, -DRB1, or -DQB1 mismatches on grade III-IV aGVHD.

Regarding the role of specific allelic level matching, matching at HLA-A, -B, -C and -DRB1 is often the most important regarding overall outcomes (improved overall survival and lower risk of GVHD). A large retrospective analysis of 334 subjects who underwent a non-T cell depleted transplant, found no difference in incidence of acute GVHD whether there was 0 or 1 mismatch in the HLA-A,-B, -C, -DRB1 and DQB1 alleles, (**Figure 5**) though a slight decrease in overall survival was seen with each increased degree of mismatch. Notably, most data, including this study, are limited to non-T cell depleted transplants, where the risk of GVHD and associated complications are inherently higher(158). In another recent review evaluating T-cell replete transplantation for adults with AML, amongst 624 subjects who received HLA-identical sibling transplants; 1193 who received 8/8 HLA-matched unrelated donor products and 406 who received 7/8 HLA-matched unrelated donor products, the overall survival and leukemia survival at 3 years after transplant did not significantly differ between the groups(159). In a prospective study that

evaluated the role of anti-thymocyte globulin (anti-T cell therapy used as one form of T-cell depletion) on outcomes between those who did and did not have a single antigen mismatch, showed no difference on overall survival or GVHD with a single antigen mismatch(160).

In summary, unrelated volunteer donors utilized in our study will be at least 9/10 allele matched, allowing for only a single locus mismatch at HLA-A, B, C, DRB1 and DQB1 loci by high resolution typing. The literature supports that despite higher rates of GVHD in the MUD setting, overall outcomes are the same due to decrease in relapse in the hematologic malignancy population. 9/10 donors who are mismatched at DQB1 will be preferentially chosen, if available, over mismatches at other loci.

### 1.2.5 T Cell Depletion in Allogeneic Stem Cell Transplantation

TCD of the donor graft has been widely established as an effective means of reducing incidence and severity of aGVHD as well as cGVHD (161). Current methods of graft manipulation are based primarily on removing T-lymphocytes by a variety of antibody-mediated approaches or physical methods of separation. CD34 selection as a method to accomplish TCD also eliminates other populations of cells (eg, B cells, monocytes, dendritic cells, and others). The loss of the latter may potentially weaken engraftment, immune reconstitution, and potentially the GVT effect, but also greatly diminishes the risk of EBV-associated lymphoproliferation, and could increase the capacity for the host to support NK cell expansion (discussed below). Although TCD has been associated with increased relapse rates in CML and AML (162), other studies have not reported increased risk of relapse following TCD alloHSCT (163, 164). This may be partly due to disease type or other factors. In the proposed study, the use of PBSCs instead of bone marrow, and the use of donor NK cells infusions (NK-DLI) following a TCD alloHSCT, may help to ameliorate these potential adverse effects, while preserving effective GVHD prevention (165).

Currently, the source of stem cells in transplantation practice is shifting from bone marrow to PBSCs. In unrelated alloHSCT, among patients younger than 20 years, marrow was used for 42% of unrelated donor transplants in 2003-2006 compared to 59% in 1999-2002. Among adults older than 20 years, marrow accounted only for 28% of unrelated donor transplants in 2003-2006 compared to 66% in 1999-2002 [CIBMTR 2007 summary data]. Neutrophil recovery is more rapid following allogeneic PBSCT, as compared with BMT. Moreover, a faster recovery of T cells has also been observed following unmanipulated PBSCs (166). Adequate recovery of T-cell and B-cell function is considered essential for long term event-free survival following alloHSCT, because lack of adequate recovery lymphocyte subsets have been associated with infectious morbidity beyond the first 100 days post-transplant (165, 167).

Increasingly, more centers are utilizing TCD-PBSCs in both the myeloablative and RIT settings. Earlier comparative studies have addressed the recovery of T cells and their functional ability following TCD-PBSCT. No significant differences in immune reconstitution between unmanipulated and partial TCD-PBSC in the HLA-matched sibling setting were demonstrated by either study. The authors suggested that with partial TCD, GVHD may be prevented, but without an apparent increased risk of reduced immune function and opportunistic infections (168, 169).

More recent studies, including one study from the ETIB in the NCI have reported the outcomes using TCD-PBSC in a RIT setting (2). Eighteen patients underwent reduced-intensity conditioning followed by alloHSCT using a TCD HLA-matched sibling allograft. All evaluable patients (n = 17) engrafted with no late graft failures. At day +28 post-RIT, 12/17 patients showed complete donor chimerism. Full donor chimerism was subsequently achieved in all patients by day 100 after

planned DLIs. These data reflect the importance of host immune status prior to RIT and suggest that targeted host lymphocyte depletion can facilitate the engraftment of TCD allografts (2).

Similarly, TCD PBSC grafts were shown to accomplish full engraftment after a RIT regimen following haploidentical alloHSCT at St. Jude's Children's Hospital. Notably, patients also showed rapid recovery of CD3(+) T-cells, T-cell receptor (TCR) excision circle counts, TCRβ repertoire diversity and NK cells during the first 4 months post-transplantation. These results were comparable to those results from a group of patients treated concurrently with a myeloablative conditioning regimen. (170). Similarly, in 2008, a German group reported outcomes of 29 patients (median age=42) using a RIT and a TCD haploidentical graft following a preparative regimen of fludarabine (150-200 mg/m$^2$), thiotepa (10 mg/kg), melphalan (120 mg/m$^2$) and OKT-3 (5 mg/day, day -5 to +14) and no posttransplant immunosuppression. Engraftment was rapid with a median time to >500 granulocytes/μL of 12 (range, 10-21) days, >20,000 platelets/μL of 11 (range, 7-38) days and full donor chimerism after 2-4 weeks in all patients. Incidence of grade II-IV degrees GVHD was 48% with the majority having grade II GVHD n=10. TRM in the first 100 days was 6/29 (20%) with deaths due to idiopathic pneumonia syndrome (n=1), mucormycosis (n=1), pneumonia (n=3) or GVHD (n=1). This study shows that in a very high-risk population, engraftment was easily achieved and the transplant was fairly well tolerated in a TCD-alloHSCT with a reduced intensity regimen using a haploidentical donor (171).

In 2008, Novitzky et al. reported on 15 patients with SAA receiving RIT and TCD PBSC grafts from HLA-matched sibling donors. Conditioning consisted of fludarabine 30 mg/m$^2$ daily for 5 days followed by CY 60 mg/kg on 2 consecutive days. The authors found overall low toxicity and rapid engraftment despite TCD, concluding that this approach does not compromise engraftment and seems effective for preventing GVHD (172). Another pediatric study, which analyzed 18 consecutive patients aged 5.5-24 years with both malignant and nonmalignant hematologic disorders, receiving TCD PBSCT from alternative donors. The preparative regimen included single dose total body irradiation (450 cGy), fludarabine (150 mg/m$^2$) and cyclophosphamide (40 mg/kg). Immunosuppression included ATG and tacrolimus. Grafts were filgrastim, CD34+, TCD PBSCs in 15 patients and TCD marrows in three patients. All 18 patients engrafted with 100% donor chimerism; only one patient developed GVHD. Immune reconstitution was achieved at approximately 6 months post-transplant for most patients. These examples illustrated encouraging results of TCD transplants from alternative donors using fludarabine-based cytoreduction in 18 high-risk patients with hematologic disorders, with no evidence of rejection and minimal GVHD (173).

**Table 2) Summary of T Cell Depletion in AlloHSCT**

| Preparative Regimen | Stem Cell Source | GVHD Prophylaxis | CD 34+ Cells/kg | CD 3 + Cells/kg | Rates of aGVHD % | Rates of aGVHD (III-IV)% | Author |
|---|---|---|---|---|---|---|---|
| RISCT | PBSC | CSA | 7.6e6 | 1e5 | 59 (II-III) | n/a | Bishop[2] |
| Myeloablative | BM | ATG/MP | n/a | n/a | 5 (I) | 0 | Papadopoulos[48] |
| Myeloablative | PBSC | CSA | 5.8e6 | 1e5 | 54 (II-IV) | 15 | Montero[49] |
| RISCT | PBSC | MMF | 17.4e6 | 1.4e5 | 38 (II-III) | 9 | Chen[55] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| RISCT | PBSC | MMF | 7.6e6 | 4.4e4 | 48 (II-IV) | 14 | Bethege[56] |
| RISCT | PBSC | CSA | 4.66e6 | n/a | 0 | 0 | Novitsky[57] |
| RISCT | PBSC/BM | Tacro | 10e6 | 3e3 | 6 (II-III) | n/a | Chaudhury[58] |
| Myeloablative | PBSC/BM | None | 14e6 | 2.7e4 | 0 | 0 | Aversa[62] |
| Nonmyeloablative | PBSC | None | 10.7e6 | 1e5 | 0 | 0 | Elhasid[64] |

*total body irradiation (TBI) containing regimen; RISCT – reduced intensity stem cell transplant; BM – bone marrow; PBSC – peripheral blood stem cells; ATG – antithymocyte globulin; MP – methylprednisolone; CSA – cyclosporine; MMF – mycophenolate mofetil; Tacro – tacrolimus; n/a – not available

### 1.2.6 GVHD prophylaxis after T cell depleted AlloHSCT

With constantly improving methods of depleting T cells from the stem cell graft in use, the amount of residual T cells capable of causing GVHD is diminishing. In recent years, most groups preferred positive selection of stem cells as better and more reliable means of TCD as compared to lectin agglutination or antibody purging of T cells(174). Given that the drugs used for GVHD prophylaxis are immunosuppressive, and potentially deleterious to NK cell function, there is great incentive to minimize usage of these agents up front. Particularly with the use of nonmyeloablative preparative regimens, the reduced degree of host inflammation may reduce the chance of GVHD developing, making GVHD prophylaxis in the setting of TCD not necessary. The precedent of withholding GVHD prophylaxis after TCD alloHSCT has been realized in multiple studies of patients with malignant diseases, including pediatric patients, receiving both HLA-matched or haploidentical HSCT. Due to the heterogeneity of the conditioning regimens between these case series, direct comparisons of the optimal regimen between studies are difficult, but are nonetheless informative.

In a study by Aversa and colleagues, 17 pediatric and adult patients with end-stage chemoresistant leukemia received TCD HSCT of a combination of BM with filgrastim-mobilized PBSCs from HLA-haploidentical family members. The conditioning regimen included TBI, ATG, cyclophosphamide and thiotepa. No post-transplant GVHD prophylaxis was used. One patient rejected the graft and the other 16 had early and sustained full donor-type engraftment. One patient who received a much greater quantity of T lymphocytes than any other patient died from grade IV aGVHD. There were no other cases of GVHD greater than or equal to grade II (175).

The same group went on to demonstrate in future studies of haploidentical HSCT with TCD that post-transplant GVHD prophylaxis could be withheld without worsening rates of engraftment or GVHD (176-178). These studies show that GVHD and graft failure, which limit the use of full-haplotype mismatched bone marrow transplants, can be overcome in both adult and pediatric patients with leukemia with reliable, reproducible CD34+ cell purification, high engraftment rates, and prevention of GVHD.

In 2007, Elhasid et al. used TCD through high-dose CD34-cell infusion in 16 pediatric patients with matched related donors for nonmalignant diseases. No GVHD prophylaxis was given. All patients engrafted, with no graft rejections. All patients survived at a median of 3 years post-transplantation, and only one developed cGVHD(179).

Thus, TCD has been safely performed in both children and adults with leukemia and nonmalignant diseases in the setting of no post-transplant prophylaxis. While the methodology of TCD and conditioning regimens vary from study to study, engraftment with low rates of GVHD are achievable.

## 1.3 PRE-CLINICAL MODELS OF NATURAL KILLER CELLS: SUSCEPTABILITY OF PEDIATRIC TUMORS TO NK CELL LYSIS

Our understanding of the biology of NK cells has increased dramatically in the last 20 years. NK cells are lymphoid cells that lyse virally infected cells and tumor cells. They express a variety of activating and inhibitory receptors that regulate their function in vivo. The relative contribution of activating versus inhibitory signals in controlling NK cell reactivity remain unclear, but likely relate to both the status of the NK cell (e.g. activated vs resting) and the status of the target cell (presence of ligand for activating receptors and/or presence or MHC or killer immunoglobulin-like receptors (KIR) ligands). Activating receptors are not expressed by normal cells but are upregulated expressed on stressed, neoplastic and virally infected cells. The major family of inhibitory receptors, called KIR, binds self-MHC class I, which is expressed on almost all nucleated cells, and inhibit NK cell function. Both virally infected cells and tumors tend to downregulate their MHC class I expression, making them susceptible to NK cell lysis.

### 1.3.1 Leukemia Models

Most of the initial studies that elucidated the basic biology of NK cells involved studying their role in eliminating leukemic cells in both in vitro and animal models of cancer. To this day, the gold standard for demonstrating a positive control of NK cytotoxicity is to use the well- characterized K562 cell line, an erythroleukemia, as a target(1-3). The following studies highlight some of the examples that illustrate how NK cells recognize and destroy hematologic malignancies through a variety of effector mechanisms.

The first NK cell line to make it to clinical trials, NK-92, has features of human activated NK cells and shows in vitro and in vivo activity against primary patient-derived leukemic target cells as well against leukemia cell lines(4). In this study, survival times of severe combined immunodeficient (SCID) mice bearing the sensitive leukemias were significantly prolonged by adoptive cell therapy with NK-92 cells. Based on this selectivity in follow up work, the potential of NK-92 cells for adoptive therapy is currently being investigated in phase I/II clinical studies(5).

As mentioned earlier, it is believed that the function of NK cells is governed by summarizing the signals by activating and inhibitory receptors(6). One method that has been examined to tilt the balance toward activation has been to block inhibitory receptors with monoclonal antibodies or small molecule inhibitors. Indeed this has been demonstrated to enhance antileukemic activity both in vitro and in vivo against AML (7-9), mastocytoma(9) and lymphoma(8, 10). NK cells can also use their activating receptor, NKG2D, to target both leukemic and lymphoma cell lines (11). Present studies are exploring ways to improve activating signals on NK cells, and one method is to engineer chimeric antigen receptors (CARs), which combine the targeting capability of an antibody to the cytotoxic potential of an effector cell, onto the surface of NK cells, leading to enhanced cytotoxicity against leukemias. For example, modified NK cells carrying a CAR against CD20, which is expressed on the surface of mature B cell leukemias and most B-cell lymphomas, displays markedly enhanced cytotoxicity in vitro. CD20-specific NK cells also efficiently lysed CD20-expressing, but otherwise NK-resistant, established and primary lymphoma and leukemia

cells(12). In addition, genetically modified NK-92 cells expressing a CAR specific for the tumor-associated ErbB2 (HER2/neu) antigen, which is overexpressed by many tumors of epithelial origin, at low effector-to-target ratios specifically and efficiently lysed established and primary ErbB2-expressing tumor cells that were completely resistant to cytolytic activity of parental NK-92 cells(13).

NK cells can utilize a variety of effector mechanisms to eliminate target cells. Virtually every cytotoxic pathway has been verified using a leukemia cell as the target. NK cells can target and eliminate ALL cell lines and primary samples coated with human anti-FLT3 monoclonal antibodies through ADCC, resulting in prolonged survival and/or reduced engraftment of several ALL cell lines and primary ALL samples in nonobese diabetic (NOD)/SCID mice(15). Bortezomib is a proteasome inhibitor that has direct antitumor effects, but can also sensitize tumor cells to killing via the death ligand, TRAIL, which is expressed on NK cells. Increases in tumor killing were only observed using perforin-deficient NK cells in one study, and thus the increased killing was found to be dependent on both TRAIL and FasL(19). In a murine model of Philadelphia chromosome-positive ALL, mice depleted of NK cells were no longer protected when challenged with the leukemia(14).

IL-2 has been extensively examined to promote clinical T and NK cell responses. While in vivo administration of IL-2 leads to activation and expansion of NK cells, IL-2 also expands immunosuppressive regulatory T cells (Tregs). Prior depletion of Tregs from IL-2 administration has led to improved antitumor effects, and in vitro data correlates with subsequent in vivo survival of leukemia-bearing mice, in which co-treatment of IL-2 with anti-CD25 (the high affinity IL-2 receptor expressed on Tregs) led to significantly improved survival compared with mice treated with either IL-2 alone or with Treg depletion(16).

Some of the earliest observations of the potential antileukemic activity of NK cells came from murine models of allogeneic HSCT. Allogeneic NK cells given as a DLI after HSCT result in a potent GVL effect(17). Efforts to enhance this effect by giving IL-2 and IL-15 showed that recipients of a NK cell infusion had lower clinical GVHD scores and suffered less severe GVHD-associated weight loss, showed accelerated lymphoid immune reconstitution as well as improved survival after leukemia challenge(18). Clinical trials of allogeneic NK cell infusions will hopefully verify the observations of preclinical leukemia and lymphoma models.

1.3.2 Neuroblastoma (This study is no longer enrolling patients with solid tumors as of Amendment K [see section **1.4.1.3**])

Neuroblastoma is the most common extracranial solid tumor in children, and was one of the first pediatric solid tumors explored in preclinical models using NK cell-based immunotherapy. Freshly isolated neuroblastoma cells lack surface expression of MHC class I, making them ideal targets for circulating NK cells(180, 181). NK cells isolated from patients have shown cytotoxicity *in vitro* against a variety of neuroblastoma cell lines(182, 183) and in a xenograft model(184). To date however, no clinical trial of adoptive NK cell transfer has been performed in neuroblastoma patients(7).

The ganglioside GD2 is expressed on neuroblastoma and has been an attractive target for immunotherapy(181). The humanized 14.18 moAb has been conjugated to interleukin-2 (IL-2) (hu14.18-IL2) in an attempt to increase NK activation resulting in enhanced antibody-dependent cellular cytotoxicity (ADCC)(185). While NK cells alone do not cure mice with neuroblastoma, another group using the chimeric anti-GD2 moAb ch14.18 combined with NK cells cured 70% of

mice if the treatment was started 7 days after tumor challenge, whereas waiting 21 days diminished the benefit(186).

### 1.3.3 Ewing sarcoma

Ewing sarcoma is the 2nd most common malignant bone tumor in children. Preclinical studies have demonstrated that Ewing sarcomas express ligands for activating receptors on NK cells, and that these receptors are critical for allogeneic NK-mediated lysis of Ewing sarcoma(187). Notably, even chemoresistant Ewing sarcoma cell lines are susceptible to NK-mediated lysis(182). Yet while tumors tested from Ewing sarcoma patients at diagnosis are resistant to NK cell lysis, the efficacy of NK cell lysis can be enhanced with IL-15(187, 188). In addition, Ewing sarcoma cell lines express heat shock proteins on their surface whose expression can be upregulated by nonlethal heat shock and targeted by NK cells, resulting in tumor lysis(189).

### 1.3.4 Rhabdomyosarcoma

Rhabdomyosarcoma is the most common soft tissue sarcoma in children. One of the earliest preclinical studies of metastatic rhabdomyosarcoma demonstrated that chemotherapy can negatively affect the efficacy of NK cells in controlling pulmonary metastases(190). NK cells were also demonstrated to be active against a patient-derived murine cell line(182). Further work also demonstrated enhanced control of rhabdomyosarcoma *in vivo* when combining cyclophosphamide with IL-15, resulting in NK cell-specific cytotoxicity of the tumor(191). In addition, mice inoculated with doxorubicin-resistant rhabdomyosarcoma show improved outcomes following adoptive NK cell therapy(192). In one clinical trial with IL-2 given to children with refractory sarcomas, one patient with rhabdomyosarcoma with lung nodules and a retroperitoneal mass had transient (<1 month) evidence of greater than a 50% shrinkage of pulmonary lesions(193).

### 1.3.5 Natural killer cells and AlloHSCT

NK cells can kill their targets through a variety of mechanisms. They express CD16, the Fc receptor, which allows the NK cells to participate in ADCC. They secrete the cytokine gamma interferon, which activates macrophages and T cells to destroy target cells. NK cells also have granules that contain perforin, which creates pores in cell membranes, and enzymes called granzymes, which enter the pores and induce apoptosis of target cells. Lastly, they also express Fas ligand and TNF-related, apoptosis-inducing ligand (TRAIL), which induces apoptosis in those cells that express the appropriate receptor[1].

Allogeneic NK cells administered or reconstituted from MHC mismatched donors can potentially induce alloreactivity if the MHC mismatch is such that inhibitory receptors are not ligated and therefore do not inactivate the NK cell. In a landmark study that retrospectively examined HSCT from a haploidentical donor to patients with refractory AML, recipients that were missing KIR ligands had a lower relapse rate, reduced GVH) and better DFS[3]. This analysis was based on the MHC of the donor and recipient, without investigating the donor KIR genotype or phenotype. The results were consistent with earlier studies in murine models that demonstrated a similar capacity for NK cells to control leukemia, as well as a lack of GVHD despite NK cell reactivity and enhanced engraftment with NK cell transfer. These features of NK cell biology: namely the ability to attack malignancy, facilitate engraftment and the inability to induce GVHD has led to great interest in utilizing NK cells within the context of alloHSCT.

Several retrospective studies explored whether the benefits of a KIR mismatch were also observed following unrelated donor HSCT. Some large registry studies failed to show a survival benefit following unrelated transplantation from KIR ligand mismatched donors[4-6], although some studies did observe benefit[7,8]. These contradicting results highlighted the complexity of the issues at play when one tries to analyze the effects of NK mediated GVT in the setting of multiple other critical variables that influence both clinical outcome and NK biology. For instance, results now suggest that TCD of the stem cell graft increases the chance that an NK mediated GVT effect will be observed, and several factors likely contribute to this. First, TCD may allow for enhanced NK cell reconstitution and proliferation due to less lymphocyte competition for homeostatic cytokines[9,10]. Second, an absence of GVHD (which is reliably prevented by TCD) may facilitate NK cell reconstitution and/or the immunosuppressive prophylaxis required for patients who receive TCD may limit effective NK reconstitution[12]. For example, CSA may have a detrimental effect on NK cell proliferation in vitro.[13] Furthermore, other critical variables, such as stem cell dose,[11] and leukemia type also influence the chance that a beneficial effect of NK mismatch may be observed and are not controlled in such retrospective studies. In adult patients, allogeneic NK cell activity seems to produce better DFS for AML than for ALL. However, data from an Italian group suggests that ALL may be the better target in pediatric leukemias. Biological support of this observation is given by a recent paper documenting that in pediatric recipients of a haploidentical HSCT, donor-derived alloreactive NK cells are generated and persist for many years(194).

IL-15 has been found to play a major role in NK cell homeostasis. IL-15 is instrumental in NK cell differentiation, peripheral expansion and survival (195). Murine models have shown enhanced NK cell function after IL-15 administration in tumor-bearing mice and IL-15 transgenic mice resulting in increased antitumor activity (195). One approach to improve cell survival, and to optimize the cytolytic potential of the NK cells in vivo, is to capitalize on the cytokine milieu in the recipient immediately following transplant, when IL-15 levels are increased (**Figure 6**). In 2008, Boyiadzis and Hakim (ETIB/NCI) published results of analysis showing the up-regulation of NK cell activating receptors following alloHSCT using a similar lymphodepleting reduced intensity regimen (as proposed in this current study) is associated with elevated IL-15 levels. They found that NK cells recover rapidly post transplant, reaching a peak at 1 month. This peak was directly related to IL-15 levels, which peaked on the day of transplant and remained high within the first 2 weeks following alloHSCT. The intent of this study is to therefore infuse NK cells early post transplant to capitalize on the natural surge of IL-15 in the early days post alloHSCT (195).

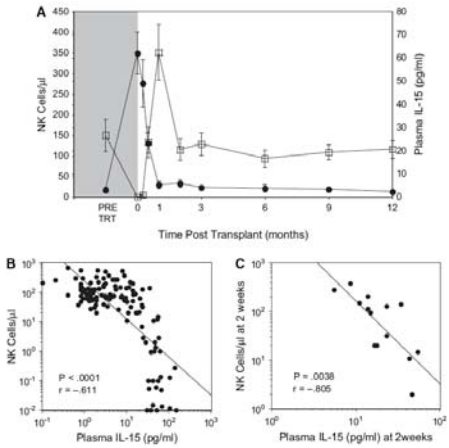



**Figure 6***)* Taken from Boyiadzis M. BBMT 14:290-300 (2008): **Correlation of NK cell recovery with IL-15 during the post-transplant period. A) Time courses of NK levels (cells/ml) (white squares) and plasma IL-15 levels (pg/ml) (black circles). B) Inverse relationship overall between NK cell numbers and plasma IL-15 levels at all time points in the first year. C) Strong inverse correlation between NK cell numbers and plasma IL-15 levels at 2 weeks post-transplant, a time when NK populations were rapidly recovering and IL-15 levels varied over a 10-fold range.**

The optimal cell dose and timing of NK needed to achieve expansion and potentiate tumor kill is unknown. On this protocol, we intend to give NK cells on days 1, 7, and 14 in order to capitalize on the post-transplant environment, including elevated IL-15 levels, which favors in-vivo NK cell expansion. The proposed divided schedule (3 weekly infusions) has been designed to maximize cell dose and potentially decrease risk of toxicity.

### 1.3.6 Natural killer cells and engraftment

In addition to contributing to anti-tumor responses, the other major potential benefit of adoptively transferring NK cells is promotion of engraftment. Preclinical mouse models have demonstrated that NK cells produce IL-1β, IL-6, G-CSF and GM-CSF, leading to enhanced engraftment of particularly granulocytic and megakaryocytic lineages (196, 197). In vitro assays of bone marrow cells co-cultured with NK cells, or NK cell supernatants, demonstrate increased hematopoietic progenitor colony formation (196). In vivo models of syngeneic and allogeneic BMT have corroborated these findings. Activated NK cells adoptively transferred to syngeneic mice results in an increase in hematopoietic progenitors in the spleen and bone marrow (198). In vivo depletion of NK cells can reverse this effect (199). Activated NK cells administered with allogeneic bone marrow cells also enhances the rate and degree of engraftment, as well as B cell reconstitution, likely through the production of cytokines as well as the elimination of the host effector cell's ability to reject the graft (196). In this study, GVHD was not observed despite using a T cell-replete graft and a complete MHC-disparate BMT. Thus, both in vitro and in vivo data suggests that NK cells may accelerate the rate and degree of engraftment, and emphasizes the importance of having activated NK cells present early during the initial stages after BMT.

### 1.3.7 EXAMINING KIR RECEPTORS

While the initial observations of the potency of NK cell alloreactivity examined donor and recipient MHC, another approach is to examine the repertoire of donor KIRs and recipient MHC. KIR expression by donor NK cells can be tested on the DNA level through genotyping or on the protein level by flow cytometry. By HLA typing the recipient, one can determine if the patient expresses receptors for the donor expressed KIR molecules expressed by the donor, making them susceptible to NK cell lysis. Patients with AML or myelodysplastic syndrome had significantly less relapse if they lacked an HLA ligand for the KIR present in the donor(200). Since the donor–recipient pairs in this study were HLA-identical siblings, simply examining donor and recipient MHC would have predicted no NK alloreactivity.

### 1.3.8 Adoptive immunotherapy with NK cells

Donor lymphocyte infusions after allogeneic HSCT can induce durable remissions in CML, and is also a treatment for CMV and EBV infections. However, DLI may exacerbate GVHD after allogeneic HSCT. Preclinical and clinical results discussed above suggest that adoptive transfer of allogeneic NK cells may be a means of inducing antitumor effects without causing GVHD. Indeed allogeneic NK cells given as a salvage therapy for AML patients can induce remission(201-203). Miller and colleagues treated 43 patients bearing either advanced solid malignancies or poor prognosis AML with immunosuppressive conditioning (high versus low dose) followed by haploidentical NK cell infusions along with IL-2 ($1.75 \times 10^6$ IU/m$^2$) for 14 days in a non-transplant

setting. Only the high-dose immunosuppressive regimen (60mg/kg intravenous cyclophosphamide and 25mg/$m^2$ intravenous fludarabine) allowed *in vivo* survival and expansion of donor-derived NK cell populations. Briefly, NK cells were isolated from CD3-depleted PBMCs, incubated overnight in X-VIVO 15 medium (2 X $10^6$ cells/mL) and 1000 U/mL IL-2 (Proleukin; Chiron, Emeryville, CA), and then administered by intravenous infusion. Doses were up to 2 x $10^7$ cells/kg dose. Three patients with melanoma and 1 with renal cell carcinoma demonstrated stable disease on restaging CT scan performed approximately 6 weeks following NK infusion and subsequently received a second course of treatment.

Grade 2 and 3 anemia was observed in 7 and 6 treatment courses, respectively, although 7 of these patients had grade 2 anemia at study entry. Two patients experienced grade 4 neutropenia and 2 others developed grade 3 neutropenia following cyclophosphamide. There were no episodes of neutropenic fever and thrombocytopenia was not observed. Hematologic toxicity was transient and there was no occurrence of prolonged cytopenia or marrow aplasia. A maximum tolerated dose was not reached and patients received the largest cell dose achievable during a single lymphapheresis collection. Five out of 19 AML patients achieved complete remission and, although only 4/19 AML patients had donors with a KIR-ligand mismatch, 3/4 were among those with complete remission. There were no adverse reactions during or after the cell infusion and GVHD was not observed(201). Of note the patients who received a second infusion demonstrated disease progression 4 to 6 weeks after the second infusion. Current studies indicate that chemotherapy prior to NK cell infusion enhances NK engraftment and in-vivo expansion. Using NK cells after HSCT is attractive since the lymphopenic environment generated by the conditioning regimen would increases systemic levels of IL-15, which is critical for NK cell development.

The experience by Miller et al was not undertaken following HSCT, but more recently there is increasing interest in incorporating NK cell infusions in this setting, and the current approach used by Miller is the administration of NK cells following HSCT (Miller, personal communication). As noted above, NK cells do not increase the risk of acute GVHD in animal models(204-206). Several studies using this approach have been recently presented at the 2008 American Society of Hematology (ASH) Annual Meeting. These studies showed that NK cell infusions following alloHSCT can be given safely and potentially provide anti-tumor effects (207-210). Furthermore, NK cell infusions may promote engraftment, decrease the incidence of aGVHD, and decrease infectious complications when given early or co-infused at the time of transplant (207, 210).

Currently there are over 30 clinical trials listed on www.clinicaltrials.gov examining the use of NK cell infusions in the autologous, haploidentical and allogeneic settings. Trials are open in both pediatrics and adult patient populations, for solid tumors (including neuroblastoma) and hematopoetic malignancy (AML, ALL, and lymphoma). Protocols include regimens where NK cells are given alone (with or without lymphodepletion) or in combination with chemotherapy or monoclonal antibody therapy or following alloHSCT.

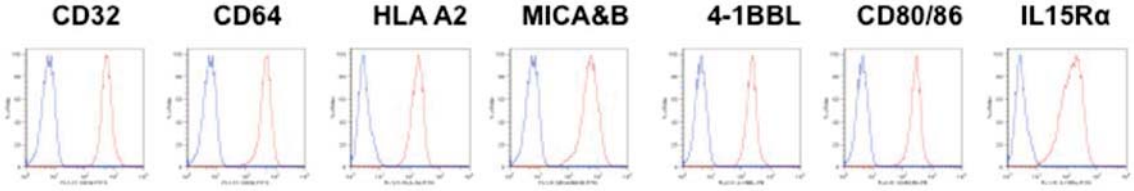
### 1.3.9 Ex-vivo Expansion of NK Cells

Essentially all clinical studies reported thus far with adoptive NK cell therapy have utilized resting NK cells purified from peripheral blood with or without incubation in rhIL-2. Handgretinger et al. incorporated ex vivo culture with rhIL15 [Handgretinger, personal communication]. These approaches incompletely activate NK cells and lead to modest expansion in number approaching a maximum of 10-fold expansion. More recently, several groups have begun utilizing cell-based

artificial antigen presenting cells to more efficiently activate and expand NK cells ex vivo. Campana and colleagues reported the use of a genetically modified cell line (the leukemia cell line K562 transduced with CD137L and membrane-bound IL-15) to activate and expand human NK cells. A median expansion of more than 20-fold was observed after 7 days of culture, with no preferential expansion of any NK cell subset(211). The NK cells generated in this culture system express gene expression profiles that are different than those expressed by primary or IL-2-activated NK cells. Expanded NK cells showed a high cytotoxicity to myeloid leukemias and even some pediatric solid tumors, including Ewing sarcoma, rhabdomyosarcoma and neuroblastoma.

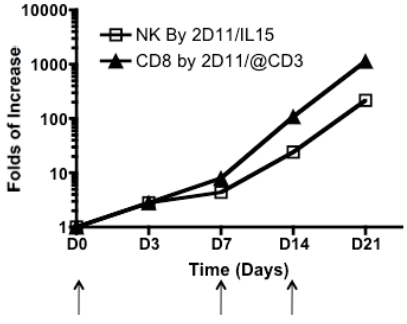
We have conducted preclinical work with KT.64.41BBL, a CD137L expressing artificial antigen presenting cells that were generated using good manufacturing practice (GMP). This cell line is designated KT.64.41BBL and can be used either to expand CD3+ T cells (when incubated with either anti-CD3 or HLA-A2 binding peptides) or to expand CD3– NK cells. For the purpose of this protocol, KT.64.41BBL cells will be used to expand CD3– NK cells collected via apheresis from the peripheral blood of donors. The expression of important costimulatory molecules for NK cells expansion on KT.64.41BBL as shown below:

**Figure 7**



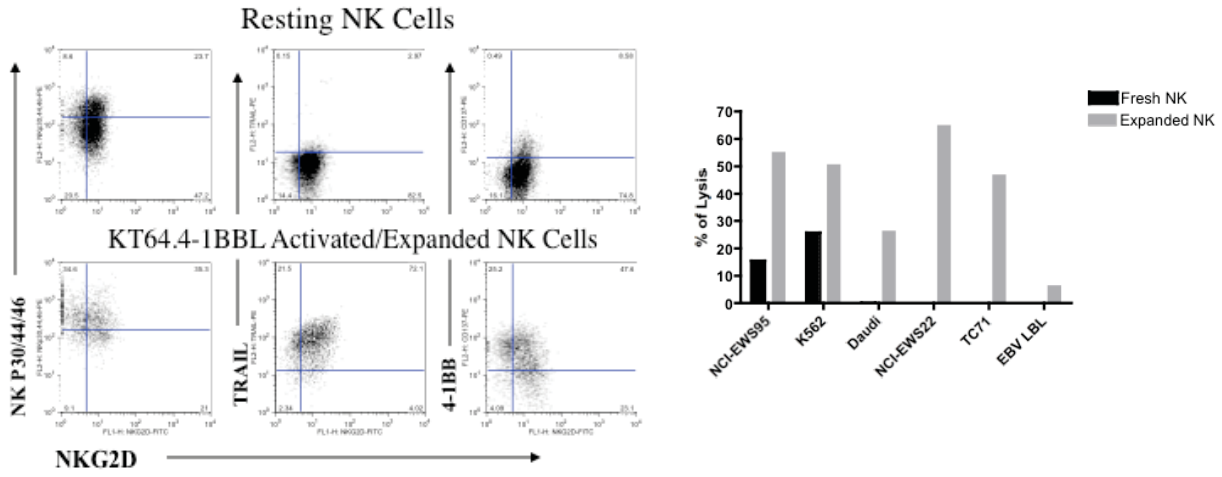

**Cell surface expression on KT64.41BBL. Flow cytometric analysis of cell surface molecules expressed on KT64.41BBL. The parent cell line is K562 that has been stably transduced with lentiviral vectors encoding CD64, HLA-A2 and 4-1BBL. The other molecules are expressed naturally by this cell line.**

**Figure 8**

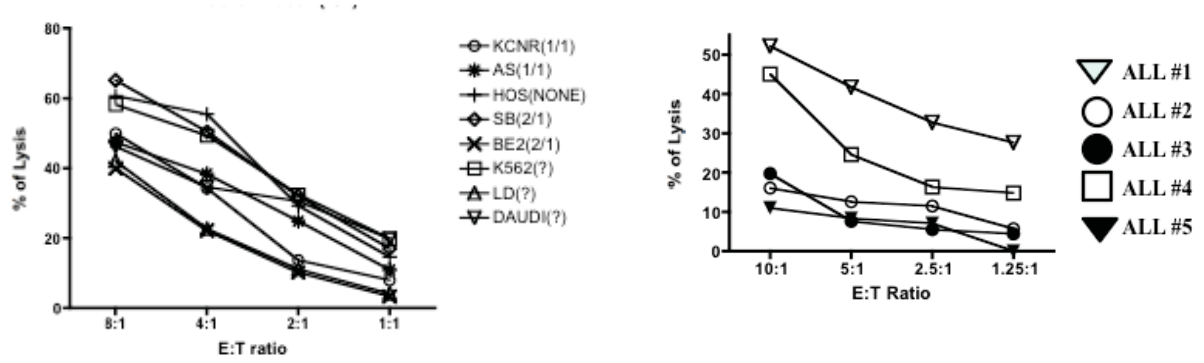



**NK cell expansion following weekly co-culture with KT64.4-1BBL + rhIL15.**

Co-incubation of KT64.4-BBL with PBMC or purified NK cells leads to expansion of NK cell numbers and activation of the cells. Using co-cultures with 1:1 NK cells:APC ratios, expansions of 2-3 logs can be accomplished over 14-21 days. The expansion is enhanced by the addition of rhIL15 to the culture. In addition to the substantial enhancement in NK cell number that is accomplished, the expanded NK cells show evidence for activation based upon upregulation of surface expression of activating NK receptors as well as enhanced cytotoxicity of tumors *in vitro*.




KT64.4-1BBL expanded NK cells show upregulation of NKRs, TRAIL and 4-1BB (left panel) and show enhanced killing of tumor




KT64.4-1BBL expanded NK cells show vigorous killing of fresh ALL blasts (left panel) obtained from 5 different patients as well as solid tumor cell lines (right panel) comprising ESFT cells (SB, LD, AS), osteosarcoma cells (HOS), neuroblastoma cells (BE2, KCNR), erythroleukemia (K562) and Burkitt's (Daudi). The numbers in parenthesis note the KIR group expression by the cell types. No difference in killing was observed regardless of KIR group expression.

**Figure 9**

## 1.4 SUMMARY: REDUCED INTENSITY TRANSPLANTATION WITH T CELL DEPLETED HEMATOPOETIC STEM CELL GRAFTS AND DONOR NK CELL INFUSIONS

Based on the preceding background information, we intend to employ an induction phase of lymphodepleting chemotherapy (EPOCH-F or FLAG), followed by reduced intensity T cell depleted alloPBSCT, using a preparative regimen of cyclophosphamide, fludarabine as utilized in our previous protocols (02-C-0259 and 01-C-0125). We believe that prevailing evidence predicts that we will accomplish rapid engraftment with minimal transplant related morbidity. In order to enhance antitumor effects, we will administer activated NK cell infusions early post transplant. The protocol will seek to enhance tumor free survival compared to that observed in the previous trial. In addition, we will investigate the biology associated with NK cell reconstitution and in the long term, hope to incorporate a multifaceted approach to enhancing immune mediated antitumor effects following allogeneic PBSCT for very high risk patients.

### 1.4.1 Study Updates

#### 1.4.1.1 Enrollment Updates (as of Amendment M, July 2014)

NK 01

The first patient on trial was a pilot patient as required by the FDA, this patient was to receive only 1 infusion of NK cells after engraftment following transplant. Cohort 1, therefore could not open until this pilot patient received EPOCH-F chemotherapy, preparative regimen, transplant, NK-cells followed by a 28-day observation period. Patient NK01 was enrolled in July 2011. This patient received a single cycle of EPOCH-F chemotherapy, but had rapidly progressive disease and received urgent radiation therapy for symptomatic lesions. This patient was therefore removed from study prior to transplant (no donor was collected) and the patient also never received the IND agent (NK-cells). The patient subsequently expired at home due to respiratory failure while in hospice. The cause of death was attributed to progression of his disease. An SAE was filed and reported to the NCI IRB because the death occurred within 30 days of going off of study.

NK 02:

Patient NK02 was therefore enrolled as a second pilot patient on September 9, 2011. NK02 was the first patient who received transplant (34 year old female with history of recurrent, metastatic desmoplastic small round cell tumor) and underwent EPOCH-F followed the preparative regimen and stem cell transplant without incidence. She received her peripheral blood stem cells from a 10/10 matched unrelated donor on October 17, 2011. She engrafted briskly on day +9 (October 26) and received NK-cell infusion on November 10, 2011. She was discharged on day +12. She subsequently developed rash, fevers and transaminitis, although a skin biopsy was not consistent with GVHD. Despite a small amount of diarrhea, she had no evidence of GVHD and proceeded to NK cell infusion on Day 24. Subsequently patient #1 developed significant diarrhea diagnosed as aGVHD with scoring of skin stage 4, GI stage 3, for a Modified Glucksberg score of Grade 4 acute GVHD. The patient continued to show response at the day 28 restaging but progressed at the day 100 restaging. The patient was taken off treatment on 3/14/12 due to progressive disease and died 4/27/12 (7 ½ months post transplant) of progressive disease.

NK03

Based on discussions with the FDA reviewers, patient NK03 was enrolled and treated in a similar manner to patient #1. Patient NK03 was enrolled on October 28, 2011. He received 1 cycle of

lymphodepleting chemotherapy with EPOCH-F and subsequently received his stem cell infusion on December 7, 2011 from his HLA-identical female sibling donor (NK03 Donor). He briskly engrafted and achieved neutrophil recovery > 500/uL on 12/17/11 (day + 10) and platelet recovery > 50k/uL on 12/21/11 (day + 14). The NK cell infusion was not given until after engraftment, as outlined in the protocol for the first pilot patient on study, with a 28 day follow up delay to assess for toxicity, prior to enrolling patients into cohort #1. He received a subsequent NK-Cell infusion on 12/21/2011 (day +14). This patient tolerated transplant well with no evidence of acute GVHD and no significant infectious complications. His initial re-staging at day +28 showed good disease response, however the patient subsequently progressed prior to the day 100 restaging in the CNS and chest. The patient was taken off treatment on 2/25/12 after patient withdrew in order to seek alternative therapy elsewhere. He died of progressive disease on 5/28/12, 5 ½ months post transplant.

NK04

Patient NK04, a 22 year old male with DSRCT, had several abdominal lymph nodes positive on PET scan prior to enrolling on study. He was enrolled on November 16, 2011 and received 3 cycles of EPOCH-F therapy and tolerated chemotherapy very well with continued disease response on each cycle. He then received a matched unrelated stem cell transplant on 2/1/12. He received NK DLI infusion #1 on 2/10/12 and NK/ DLI infusion #2 on 3/7/12. He developed a maximum Grade 2 acute GVHD, which responded to imunosuppression. Restaging at day 100 showed no evidence of disease. Shortly after day 100 evaluation, patient developed septic shock and acute renal failure. He was discharged home after a prolonged hospitalization and died in hospice 259 days after transplant

NK05

Patient #5, a 15 y/o male with EWS was enrolled with no evidence of disease on December 19, 2011 and received 3 cycles of EPOCH-F therapy and tolerated chemotherapy very well. He received his stem cell transplant from a matched unrelated donor on March 7, 2012. He received his first dose of NK cells on day +9 following stem cell transplant and subsequently developed rash, diarrhea and fevers, categorized as grade 4 aGVHD of the skin and GI tract. He also developed transient hypotension at that time. He was started on corticosteroids and his GVHD symptoms completely resolved within 48 hours. He was weaned from immunosuppression and received a second dose of NK cells on April 10, 2012. He subsequently developed skin rash and diarrhea in the setting of fevers and was restarted on corticsteroids, with brisk reversal of symptoms again. Patient has undergone restaging every 3 months and remains no evidence of disease, most recent visit August 2013. He is currently off all immunosuppression with no signs of GVHD and is doing well. He has since developed chronic renal failure, likely multifactorial in etiology, but related in part to HCT. Complications included development of thrombotic microangiopathy, which may have been related to immunosuppression given for GVHD. Also had a history of nephrotoxicity from prior chemotherapy. His current renal status remains stable. Most recent disease restaging evaluation at 2 years post transplant shows no definitive evidence for disease, but there is a site of concern in the first rib. This will be closely monitored with plans for restaging in 6-9 weeks.

NK06

Patient NK06 (24 y/o male with DSRCT) was enrolled on 2/16/12, and received 2 cycles of EPOCH-F chemotherapy and underwent matched related stem cell transplant on April 4, 2012. He

subsequently received his first dose of NK cells on April 12, 2012. Restaging on day 28 showed no evidence of disease, however his PET scan just prior to day 100 showed progressive disease. He received additional NK cell infusions on a single patient exemption trial approved by the FDA and NCI IRB. After further disease progression, he was taken off that study and is currently being treated at home as per primary oncologist. He continues to tolerate therapy well and has never developed evidence of GVHD.Patient has been receiving on-going therapy under the direction of his home physician; he remains without evidence of GVHD and tolerating further chemotherapy well.

NK07

NK07 was enrolled 5/4/12 and received 1 cycle of EPOCH-F therapy and tolerated chemotherapy very well. Patient NK07 had matched related stem cell transplant on 6/20/12. NK DLI infusion #1 was on 6/28/12 and NK DLI infusion #2 on 7/26/12. NK07 developed no acute GVHD nor chronic GVHD thus far. Unfortunately patient was found to have relapsed on day +100 restaging. He was treated at home as per primary oncologis and ultimately passed away from progressive disease at day + 321 post-transplant.

NK08

NK08, was enrolled 5/24/12 and received 3 cycles of EPOCH-F therapy and tolerated chemotherapy very well. Patient received his matched related stem cell transplant on 8/15/12 and his NK cell infusions on 8/23/12 and 9/19/12. Patient tolerated cellular infusions well, without complications. Furthermore, he developed no acute nor chronic GVHD thus far. Unfortunately patient was found to have relapsed on day +100 restaging and died from disease progression 607 days following HCT.

NK09

NK09 was enrolled on 7/13/12 and received 1 cycle of EPOCH-F therapy. Unfortunately, her matched sibling donor experience an anaphylactic reaction to GCSF shot for stem cell mobilization, therefore patient was taken off study secondary to no longer having a matched donor available. Patient has subsequently relapsed and is receiving alternative chemotherapy.

NK10

NK10 was enrolled on 8/17/12 and received 1 cycle of EPOCH-F therapy. Unfortunately, he had rapid progression of disease during this cycle, resulting in a partial cord compression. He was therefore taken off study in order to receive emergent radiation therapy. He died in hospice care less than 1 month later.

NK11

NK11 was enrolled on 10/25/12 and received 3 cycles of EPOCH-F therapy, which he tolerated well. He received his unrelated stem cell transplant on 1/9/13 and the first dose of NK cells on 1/18/13. Within 48 hours of NK cell infusion, patient experienced rash, fevers and mild hypotension, which responded to fluid bolus. This coincided with engraftment and was thought to reflect cytokine effects seen with engraftment, however, patient developed progressive rash and diarrhea and was diagnosed with Grade 2 aGVHD on 1/22/13 (stage 3 skin and stage 1 GI), which was confirmed by skin and GI biopsy. Patient responded well to steroid therapy, however developed reactivation of adenovirus causing a hemorrhagic cystitis and hemorrhagic nephritis. Therefore, an aggressive wean of the steroids were attempted and patient had an acute flare of his

GVHD resulting in grade 4 GVHD involving his eyes, skin (stage 4), liver (stage 1) and GI tract (stage 3). Patient's course was complicated by GI bleeding and a presumptive Steven Johnson's type reaction (secondary to antibiotics) requiring admission to the ICU. Patient's aGVHD responded to steroid therapy was well controlled with immunosuppression consisting of steroids and cyclosporine. He was found to have relapse on day + 156 and was taken off-treatment to receive alternative therapy. He died from progressive day at approximately day + 300 post-transplant.

NK12

NK12 is an 18-year-old male with DSRCT who enrolled on study on February 22, 2013. He received 2 cycles of EPOCH-F therapy, which he tolerated well. He enrolled on study with no evidence of disease and entered transplant as such. He received his stem cell infusion on April 24, 2013 from an unrelated male donor. He was the first unrelated recipient on this protocol to receive tacrolimus immunosuppression for prophylaxis. He received his first NK-Cell infusion on 5/17/2013 (day +23), and a second infusion on 6/14/2012. He tolerated both infusions well with no adverse reactions. He has done fairly well from the transplant standpoint with no evidence of aGVHD. He developed disease progression at the 1-year post-transplant evaluation (Day + 383). Is alive with disease.

NK13

NK 13 is a 13 year-old male with Ewings sarcoma who enrolled on study on March 7, 2013. He received 3 cycles of EPOCH-F therapy, which he tolerated well, however following the 3$^{rd}$ cycle he was found to have significant, recurrent disease on re-staging scans and he was therefore taken off study prior to transplant.

NK14

NK14 is a 21 year-old female with Ewings sarcoma who enrolled on study on March 21, 2013. She received 3 cycles of EPOCH-F therapy, which she tolerated well. She received her stem cell transplant from her matched sibling on June 12, 2013 and subsequently her first dose of NK cells on Day + 21 (July 3). She tolerated these infusions very well. She subsequently developed elevation of her transaminases approximately 10 days following the 1$^{st}$ NK cell infusion. She had a liver biopsy for persistent elevation of live enzymes that revealed mild GVHD. She therefore did not receive her second NK cell infusion. Her transaminases normalized after several weeks without immunosuppressive therapy. At the 1-year evaluation two new lesions (right proximal tibia and right anterior 5th rib) were found on PET scan. Subject has since undergone biopsy and results are pending. This is worrisome for disease progression.

NK15

NK 15 is a 26 year-old male with Alveolar Rhabdomyosarcoma who enrolled on study on September 18, 2013. He received 1 cycles of EPOCH-F therapy, which he tolerated well. Unfortunately his pre-transplant restaging revealed progressive disease and he was taken off study prior to transplant.

NK16

NK16 is a 23 yo M with Acute lymphoblastic leukemia who enrolled on study on November 1, 2013. He was in a complete remisison and went directly to a myeloablative transplant. He received his cells on 11/13/13 and had a rapid engraftment. He received both NK-DLI and remains in an

MRD negative complete remission at 6 months post transplant. He also has attained full donor chimerism.

NK17

NK17 is a 24 yo M with acute lymphoblastic leukemia who enrolled on study on November 8, 2013. He was in a complete remission and went directly to a myeloablative transplant. He received his cells on 11/26/13 and has had a rapid engraftment. He received both NK-DLI and remains in a MRD negative complete remission at 6 months post-transplant. He has attained full donor chimerism.

NK18

NK18 is a 25 yo F with acute lymphoblastic leukemia who was in a complete remission and went directly to a myeloablative transplant. She received her cells on 1/14/14 from her matched sibling brother and had a rapid myeloid engraftment. Unforutnately she never attained donor lymphoid engraftment and ultimately rejected her graft requiring a second conventional transplant. She is currently 100 days following a second transplant and remains in remission with evidence for grade 1 GVHD.

NK19

NK19 is a 18 yo M with acute lymphoblastic leukemia who was in a complete remission and went directly to a myeloablative transplant. He received his cells from his matched sibling sister and had a rapid myeloid engraftment. He received 1 x 10e4 CD3/kg and was treated on amendment K prior to the increased T-cell addback in the new revision. Despite initial robust engraftment, he has shown signs for loss of engraftment and is currently being reated with serial DLI.

1.4.1.2 Rationale for Two Escalation Cohorts

Based on our experience in the first 11 patients enrolled (8 transplanted), the following modifications were as per Amendment H:

1.) Separate the matched realted donor recipients and the matched unrelated recipients into two cell-dose escalation cohorts. As there has been no acute GVHD seen in the related donor recipients (0/4) and 100% aGVHD in the unrelated donor recipients (4/4), we plan to separate patients with related and unrelated donors into two cohorts and advance the NK cell dose in the related donor cohort and continue at the initial cell dose cohort for the unrelated donor recipients. In addition, we will add immunosuppression (cyclosporine) for the unrelated cohort in order to decrease the risk of acute GVHD, which although thus far is steroid responsive, it has been high grade in several unrelated stem cell recipients and has effected the ability to give additional NK cell infusions.
2.) We will delay the first dose of NK cell infusion (from day +7 to day +21), such that it does not overlap with the timing of engraftment to help decipher whether the NK cells play a role in development or severity of aGVHD.
3.) We will require that sarcoma patients must be in a state of non-measureable disease at the time of transplant, as thus far all of the patients with bulky disease have had rapid progression on the trial. This has either prevented them from going on to receive a transplant, or have had early progression post-transplant, therefore we believe the risk-benefit ration for these patients to be too great.

Additional modifications to the protocol are based on the poor accrual for patients with acute leukemias. We believe this is due to the fact that myeloablative transplant is the standard of care for these patients and reduced intensity transplant is therefore a deterant for referrals. We will keep a reduced intensity option available for those patients who are unlikely to tolerate ablation, such as some previously transplanted patients. Therefore:

4.) We will add a myeloablative preparative option for the patients with hematologic malignancies in order to increase accrual of this population – the preparative regimens are derived from current Children's Oncology Group (COG) ALL (ASCT0431) and AML (AAML1031) frontline transplant protocols.

#### 1.4.1.3 Rationale for Closing Accrual to Solid Tumors

As of Amendment K, 15 subjects have been treated on this study (see table below). Ten of these subjects have received HSCT, and all but three have experienced early progressive disease. At this time, since most of our experience has been with solid tumors, we will focus future efforts on accruing subjects with hematologic malignancies to determine the outcomes of this regimen in this patient population.

| Patient | Gender | Age | Race | Disease | Donor | NK Cell Dose | acute GVHD | chronic GVHD | Best Response following transplant +NK cells | Relapse | Status |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 22 y | C | Ewing's | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 2 | F | 34 y | C | DSRCT | MUD | 1 x 10e5/kg | Grade IV GVHD | None | PR | Day 100 | DoD day + 190 |
| 3 | M | 33 y | C | Ewing's | MSD | 1 x 10e5/kg | None | None | PR | Day 70 | DoD day + 171 |
| 4 | M | 22 y | C | DSRCT | MUD | 1 x 10e5/kg | Grade II GVHD | None | CR | NED | DoSepsis - day + 191 |
| 5 | M | 16 y | C | Ewing's | MUD | 1 x 10e5/kg | Grade III GVHD | None | Remained NED | NED | NED |
| 6 | M | 24 y | AA | DSRCT | MSD | 1 x 10e5/kg | None | None | CR/NED by PET | Day 100 | On chemotherapy |
| 7 | M | 18 y | C | ARMS | MSD | 1 x 10e5/kg | None | None | Remained NED | Day 85 | On chemotherapy |
| 8 | M | 14 y | C | Ewing's | MSD | 1 x 10e5/kg | None | None | Remained NED | Day 93 | On chemotherapy |
| 9 | F | 15 y | AA | RMS | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 10 | M | 9 y | C | Ewing's | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 11 | M | 15 y | C | Ewing's | MUD | 1 x 10e5/kg | Grade IV GVHD | None | Remained NED | Day 156 | On chemotherapy |
| 12 | M | 18 y | C | DSRCT | MUD | 1 x 10e5/kg | None | None | Remained NED | NED | NED |
| 13 | M | 13 y | C | Ewing's | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 14 | F | 21 y | C | Ewing's | MSD | 1 x 10e6/kg | Liver GVHD* | TOO EARLY | Remained NED | NED | NED |
| 15 | M | 26 y | C | ARMS | N/A | TOO EARLY | N/A | TOO EARLY | N/A | N/A | N/A |

#### 1.4.1.4 Rationale for Increasing T-cell dose ("add-back") in recipients of related donor products

Due to a recent occurrence of secondary graft rejection and based on an analysis of all subjects transplanted to date, we have identified that there is a discrepancy in CD3 donor chimerism between related and unrelated donor recipients. The median donor CD3 chimerism at day 14 in recipients of related and unrelated donors was 11% (range, 2-73%) and 93% (range, 64-100%) respectively (p=0.01). At day 28, at the median donor CD3 chimerism was 45.5% (range, 8-92%) and 100% (range, 92-100%) in related and unrelated donor recipients, respectively (p=0.006). Importantly no high grade GVHD has been observed in recipients of products from related donors.

Because of the risk of graft rejection and the increased relapse risk associated with sustained low donor lymphoid chimerism, subsequent recipients of matched related donor products will receive

a higher T-cell add back (1-2 x $10^5$ CD3/kg) in the initial stem cell product. This represents a one-log increase from 1-2 x $10^4$ CD3/kg.Since this increased T-cell dose will increase the risk of GVHD, recipients of related donor products will also receive calcinurin inhibitor for GVHD prophylaxis. This will also make the related and unrelated donor cohorts more consistent as the same GVHD prophylaxis was already instituted in the unrelated donors due to GVHD (outlined in sections **1.4.1.1** and **1.4.1.2**).

## 2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

### 2.1 ELIGIBILITY CRITERIA FOR PATIENTS (RECIPIENT)

2.1.1 Inclusion Criteria: Patient (Recipient)

2.1.1.1 Hematologic Malignancies Diagnoses:

a) Acute lymphoblastic leukemia (ALL) with a history of bone marrow relapse in clinical remission (CR) #2 or greater, or in CR#1 if prior induction failure; or with an M1 marrow if unable to achieve CR.
b) Philadelphia chromosome positive ALL patients who;
   1) Have progressed through or relapsed following TKI therapy or conventional myeloablative therapy

      OR

   2) Are ineligible to receive tyrosine kinase inhibitor (TKI) therapy **AND** myeloablative HSCT
c) Acute Myelogenous Leukemia (AML) with a history of bone marrow relapse in remission CR #2 or greater; or with an M1 marrow if unable to achieve CR; or in CR#1 if prior induction failure; or any of the following High-Risk categories:
   1) FLT3/ITD+ with high allelic ratio > 0.4 (HR FLT3/ITD+) regardless of low risk features.
   2) Presence of monosomy 7, monosomy 5, or del5q, without inv(16)/t(16;16) or t(8;21) cytogenetics or NPM or CEBPα mutations.
   3) AML without inv(16)/t(16;16), t(8;21), NPM, CEPBα mutations, monosomy 7, monosomy 5, del5q, or HR FLT3/ITD+, but with evidence of residual AML (≥ 0.1%) at end of Induction I.
d) Hodgkin's and Non-Hodgkin's Lymphoma with refractory disease or relapse after at least one salvage regimen, or after autologous stem cell transplant
e) Juvenile Myelocytic Leukemia (JMML) with <10% blasts in marrow and blood, who are not eligible for effective standard therapies.
f) Chronic Myelogenous leukemia (CML) with history of blast crisis (ALL/AML) or progressive disease failing Tyrosine-kinase inhibitor (TKI).

2.1.1.2 Age: 4 to ≤ 35 years old at the time of enrollment.

2.1.1.3 All previous cytotoxic chemotherapy must be completed at least 2 weeks prior to study entry. Any prior non-hematologic vital organ toxicity (cardiac, pulmonary, hepatic, renal) of any previous therapy must have resolved to grade 1 or less, unless specified elsewhere in Section 2.1.

**Exceptions:**

- There is no time restriction in regard to prior intrathecal chemotherapy provided there is complete recovery from any acute toxic effects; or
- Subjects receiving standard ALL maintenance chemotherapy will not require washout.

2.1.1.4 All previous immunologic or molecularly targeted therapy must be completed at least 2 weeks prior to study entry. Any prior non-hematologic toxicity of any previous therapy must have resolved to grade 1 or less, unless specified elsewhere in Section 2.1, or except BCR-ABL tyrosine kinase in patients who have Ph+ ALL, where there will be no washout period.

2.1.1.5 Prior investigational therapy must be completed at least 30 days prior to study entry.

2.1.1.6 Patients with prior autologous or allogeneic transplant are eligible. Patients must be > 100 days post transplant and have no evidence of active GVHD.

2.1.1.7 Performance status: ECOG 0, 1 or 2; or Karnofsky of ≥ 60, or for children ≤10 years of age, Lansky ≥ 60 (**Appendix 1**). Life expectancy >3 months.

2.1.1.8 Availability of HLA-matched related or unrelated donors. Related donors must be 5 or 6/6 antigen matched. Unrelated donors must be at least 9/10 allele matched.

2.1.1.9 Cardiac function: Left ventricular ejection fraction ≥45% by MUGA or ECHO, fractional shortening ≥28% by ECHO.

2.1.1.10 Pulmonary function: DLCO ≥40% of the expected value corrected for alveolar volume and hgb for reduced intensity transplant and DLCO ≥55% for myeloablative regimen. For children who are unable to cooperate for PFTs, the criterion is: No evidence of dyspnea at rest, no exercise intolerance, and no requirement for supplemental oxygen therapy.

2.1.1.11 Liver function: Serum total bilirubin < 2 mg/dl, serum AST and ALT ≤2.5 x upper limit of normal. Patients with Gilbert syndrome are excluded from the requirement of a normal bilirubin. (Gilbert syndrome is found in 3-10% of the general population, and is characterized by mild, chronic unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis).

2.1.1.12 Renal function: Age-adjusted normal serum creatinine according to the following table or a creatinine clearance ≥ 60 ml/min/1.73 $m^2$.

| Age (years) | Maximum serum creatinine (mg/dl) |
|---|---|
| ≤5 | 0.8 |
| >5 ≤ 10 | 1.0 |
| >10 ≤ 15 | 1.2 |
| > 15 | 1.5 |

2.1.1.13 Marrow function: ANC must be > 750/$mm^3$ (unless due to underlying disease in which case there is no grade restriction), platelet count must be ≥ 75,000/$mm^3$ (not achieved by transfusion)

unless due to underlying disease in which case there is no grade restriction). Lymphopenia, CD4 lymphopenia, leukopenia, and anemia will not render patients ineligible.

2.1.1.14 Ability to give informed consent. For patients <18 years of age their legal guardian must give informed consent. Pediatric patients will be included in age-appropriate discussion in order to obtain verbal assent.

a. Durable power of attorney form completed (patients ≥18 years of age only).

b. Female patients (and when relevant their male partners) must be willing to practice birth control (including abstinence) during and for two months after treatment, if of childbearing potential.

2.1.2 Exclusion Criteria: Patient (Recipient)

2.1.2.1 Uncontrolled infection.

2.1.2.2 Active CNS malignancy as defined by:

a. Lymphoma: tumor mass on CT scan or leptomeningeal disease
b. Leukemia: CNS 2 or CNS 3 classification (**Appendix 8**).

2.1.2.3 Lactating or pregnant females (due to risk to fetus or newborn).

2.1.2.4 HIV positive (due to unacceptable risk associated with severe immune suppression).

2.1.2.5 Hepatitis B surface antigen (HBsAg) positive or hepatitis C antibody positive with elevated liver transaminases. All patients with chronic active hepatitis (including those on treatment) are ineligible.

2.1.2.6 Patients who require systemic corticosteroid or other immunosuppressive therapy for GVHD. Immunosuppressive therapy must be stopped at least 28 days prior to protocol C1D1. Steroids for physiologic replacement, for ALL maintenance or topical agents and/or inhaled corticosteroids are permitted.

2.1.2.7 High risk of inability to comply with transplant protocol, or inability to give appropriate informed consent in the estimation of the PI, social work, psychiatry, or the stem cell transplant team.

2.1.2.8 Fanconi Anemia

2.1.2.9 Clinically significant systemic illness (e.g. serious active infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that in the judgment of the PI would likely compromise the patient's ability to tolerate protocol therapy or significantly increase the risk of complications.

## 2.2 ELIGIBILITY CRITERIA FOR DONORS

2.2.1 Inclusion Criteria: Donor

2.2.1.1 Weight ≥ 15 kilograms and for unrelated donors, ≥ 18 years.

2.2.1.2 HLA-matched related or unrelated allogeneic donors. Genotypically HLA identical twins may serve as stem cell donors. Related donors must be 5 or 6/6 antigen-matched. Unrelated donors must be at least 9/10 allele matched.

2.2.1.3 For donors <18 years of age, he/she must be the oldest suitable donor, their legal guardian must give informed consent, the donor must give verbal assent, and he/she must be cleared by social work and a mental health specialist to participate.

2.2.1.4 For donors ≥ 18 years of age, ability to give informed consent.

2.2.1.5 Adequate peripheral venous access for apheresis or consent to use a temporary central venous catheter for apheresis.

Donor selection will be in accordance with NIH/CC Department of Transfusion Medicine (DTM) criteria and, in the case of an unrelated donor, the National Marrow Donor Program (NMDP) standards and FDA 21 CFR 1271.

2.2.2 Exclusion Criteria: Donor

2.2.2.1 History of medical illness that in the estimation of the PI or DTM/NMDP physician poses prohibitive risk to donation including, but not limited to, stroke, hypertension that is not controlled with medication, or heart disease. Individuals with symptomatic angina or a history of coronary bypass grafting or angioplasty will not be eligible.

2.2.2.2 Anemia (Hb < 11 gm/dl) or thrombocytopenia (<100,000/μl).

2.2.2.3 Identical twins will be excluded; the lack of MHC incompatibility will alter the toxicity profile in such a way as to make the results uninterpretable.

2.2.2.4 Breast feeding or pregnant females. Donors of childbearing potential must use an effective method of contraception during the time they are receiving filgrastim. The effects of cytokine administration on a fetus are unknown and may be potentially harmful. The effects upon breast milk are also unknown and may potentially be harmful to the infant.

2.2.2.5 High risk of inability to comply with protocol requirements as determined by the principal investigator and donor center team.

2.2.2.6 Positive screening test for transfusion-transmissible infection in accordance with DTM or NMDP donation standards, including HIV-positive, hepatitis B surface antigen (HBsAg) positive or hepatitis C antibody positive.

## 2.3 RESEARCH ELIGIBILITY EVALUATION

2.3.1 Clinical Evaluation Recipient

2.3.1.1 All patients will be screened by complete medical history and physical examination. Pre-transplant history and physical documentation templates for the recipient can be found in CRIS.

2.3.1.2 Eligibility evaluation for recipient participation in this trial is outlined in Appendices 2 & 3 and must be completed within 30 days of study entry unless otherwise specified.

2.3.2 Clinical Evaluation Donor

2.3.2.1 All donors will be screened by complete medical history and physical examination. The evaluation of minor donors will be performed by a practitioner with pediatric expertise.

2.3.2.2 Donor eligibility is outlined in **Appendix 3** and must be completed within 30 days of study entry unless otherwise required by CC DTM or NMDP donation standards.

2.3.2.3 Donors at NIH will complete a Blood Bank screening questionnaire in the Department of Transfusion Medicine (DTM). NIH Donors will have an interim health assessment performed by the DTM staff immediately before each collection procedure.

2.3.2.4 For unrelated donors, all evaluations will be performed at an NMDP-approved donor center, with the exception of specified blood samples to be sent to the NIH for analysis.

2.3.3 Specialty Consultation

2.3.3.1 Dental consultation to assess need for teeth cleaning, caries correction or extraction (patients only).

2.3.3.2 Social work consultation (patient and related donor).

2.3.3.3 Durable power of attorney form completed (patients ≥ 18 years of age).

Related donors (at NIH) who are minors will be evaluated by a mental health specialist with pediatric expertise (psychologist or psychiatrist) prior to the assent process to determine willingness to participate (donors < 18 years of age only).

2.3.4 Laboratory Serologic Evaluations

****In attempt to preserve adequacy of peripheral access for apheresis, antecubital phlebotomy will be avoided when possible during donor screening*

2.3.4.1 Typing for HLA-A, -B, C and –DRB1 performed in at the NIH DTM (related donor and patient). HLA typing may be performed at any time prior to entry without time limitation. The resolution of the HLA typing must distinguish among similar alleles being inherited in the family. If an unrelated search is required, the patient will be HLA typed at allele resolution for HLA-A, B, C, DRB1, DQB1 by the C. W. Bill Young Marrow Program/ Georgetown University, 3rd Floor, 11333 Woodglen Drive, Rockville, MD 20852, 301-998-8900. Buccal swabs will provide sufficient material for this testing. Typing for unrelated donors will be performed through the NMDP Customized Typing Program according to NMDP standards (allele resolution for HLA-A, B, C, DRB1, DQB1), with source documentation at NIH.

2.3.4.2 Blood type, screen, and, if blood type identical, RBC phenotyping, must be performed at the NIH DTM laboratory (related donor and patient) or at a NMDP. In cases of major ABO incompatibility isohemagglutinin titers must be performed. Typing for unrelated donors may be performed at an NMDP Donor Center according to NMDP

standards, with source document verification at NIH. Typing may be performed at any time prior to entry without time limitation.

2.3.4.3 Leukemia Patients: Unilateral bone marrow aspirate and biopsy. Cytogenetics and flow cytometry analysis (through the NCI Laboratories of Pathology) should be performed on bone marrow aspirate if disease can be followed by those modalities. Aspiration should be repeated within 14 days prior to the first cycle of induction chemotherapy and within 14 days prior to starting transplant preparative regimen.

**OR**

Lymphoma Patients: Bilateral bone marrow aspirates and biopsies for lymphoma patients with a prior history of bone marrow involvement, if clinically indicated.

2.3.4.4 Infectious disease testing per Blood Bank/NMDP standards to include HIV, HTLV-I/II, HBV, HCV, CMV, and syphilis (donor). Donor may also have HIV/HCV nucleic acid test and West Nile virus (WNV) antigen and VZV serology screening (according to the standards of DTM Blood Bank/NMDP). Donor testing will be performed no more than 30 days prior to each collection.

2.3.4.5 CBC with differential (patient and donor). Must be repeated within 72 hours of first collection and within 24 hours of subsequent collections (donor).

2.3.4.6 Chemistry 20 (Chem 20): electrolytes, glucose, BUN, creatinine, AST, ALT, alkaline phosphatase, bilirubin (total and direct), albumin, calcium, magnesium, phosphorus, uric acid, lactate dehydrogenase (LDH), total protein, CK.

2.3.4.7 Protime (PT), partial thromboplastin time (PTT), (patient and donor)

2.3.4.8 Urine or blood βHCG in post-pubertal females (patient and donor).

2.3.4.9 24-hour urine for creatinine clearance (patient only, if creatinine is abnormal).

2.3.5 Radiologic, Nuclear Medicine, and Specialty Studies (Recipients only)

2.3.5.1 CT scans of the head, chest, abdomen and pelvis and primary tumor.

2.3.5.2 MRI scans of the head and primary tumor, if clinically indicated.

2.3.5.3 FDG-PET scan on all patients as clinically indicated. Technetium$^{99m}$ bone imaging if clinically indicated.

2.3.5.4 MIBG Scan for patients with MIBG avid neuroblastoma.

2.3.5.5 Pulmonary function tests (vital capacity, FEV-1, DLCO).

2.3.5.6 Electrocardiogram and MUGA scan or ECHO.

2.3.6 Pathologic/Tissue Evaluation

2.3.6.1 Tissue will be reviewed when available, by the NCI Department of Pathology, and the diagnosis must be confirmed prior to enrollment. When tissue is not available, reports from a CLIA certified laboratory is sufficient

### 2.4 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate and related donor with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient and donor on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

NIH UID numbers will be generated on unrelated donors while maintaining the anonymity required by the NMDP, and a completed eligibility checklist must be emailed to the CRO for registration of unrelated donors. The CRO will also be notified for removal of patients and donors from study.

## 3 STUDY IMPLEMENTATION

### 3.1 STUDY DESIGN

This will be a dose escalation study of NK-DLI infusions following alloHSCT. All patients will receive the exact same disease-specific treatment regimen from either a related or unrelated matched allogeneic PBSC and NK cell donor.

All lymphoma recipients may receive 1 to 3 cycles of EPOCH-F to achieve immune depletion and for concurrent disease control. This will be followed by a myeloablative or non-myeloablative PBSCT, as clinically indicated.

Leukemia patients with M1 marrow, but detectable minimal residual disease (MRD) by flow may receive 1 to 3 cycles of FLAG or EPOCH-F, followed by either a myeloablative or non-myeloablative PBSCT. Those patients in a CR with no evidence of MRD may proceed directly to transplant preparative regimen, however, if there is a delay secondary to donor availability, they may receive 1 to 3 cycles of FLAG or EPOCH-F chemotherapy. Those patients with poor lung function, a history of prior total body irradiation, or who under the discretion of the PI are felt to be at high risk of death from a myeloablative preparative regimen will receive a non-myeloablive transplant, regardless of disease status.

Patients will be enrolled to 3 dose levels in 2 cohorts as defined below. The first patient in each dose cohort will be ≥ 18 years of age. If the first patient does not experience DLT by 28 days after the first NK cell infusion, subsequent patients < 18 years of age may be treated with NK-DLI. If the first patient (adult) in a cohort experiences DLT, the 2 additional patients ≥ 18 years of age in that dose cohort must complete 28 days post NK infusion without further DLT, before treating pediatric patients.

*Prior to enrolling on the first cohort, a single patient 18 years of age or older will receive DLI-NK cells once ANC reaches at least 1000 cells/μL or within 42 days following transplant, whichever comes first. This will allow analysis of the potential risk for NK induced graft loss. This first patient must be monitored for DLT for at least 21 days following the last NK dose to evaluate for late toxicity prior to infusing NK cells to patients enrolled in cohort 1. In the*

*absence of DLT, the first patient may proceed to a second infusion of DLI-NK within 3 months of the initial NK infusion.*

Subsequently cohorts of patients entered onto the study will receive DLI-NK on day 21 (± 3 days). A second infusion of DLI-NK will be given on Day 49 (approximately 28 days after the first infusion) (± 7 days) if toxicity attributable to NK cells has resolved. At the completion of each dose level, the toxicities of all patients in that level will be analyzed for trends and reported to the FDA, and in the absence of DLT, accrual will proceed to the next dose level. In the absence of dose limiting toxicity, the dose of DLI-NK will be escalated by 1 log per level, as defined in the table below. Given the differences in toxicities experienced by patients receiving PBMC and DLI-NK cells from related donors versus unrelated donors, the dose levels will be escalated in two cohorts: Cohort 1: Patients receiving cells from related donors versus Cohort 2: Patients receiving cells from unrelated donors.

| Dose Level | Cohort 1: Patients with Related Donors | Cohort 2: Patients with Unrelated Donors |
|---|---|---|
| Dose Level 1 | 1 X $10^5$ NK Cells/kg infused on day 7 (± 3d), 35 (± 7d) **COMPLETED** | 1 X $10^5$ NK Cells/kg infused on day 21 (± 3d), 49 (± 7d) |
| Dose Level 2 | 1 X$10^6$ NK Cells/kg infused on day 21 (± 3d), 49 (± 7d) | 1 X$10^6$ NK Cells/kg infused on day 21 (± 3d), 49 (± 7d) |
| Dose Level 3 | 1 X $10^7$ NK Cells/kg infused on day 21 (± 3d), 49 (± 7d) | 1 X $10^7$ NK Cells/kg infused on day 21 (± 3d), 49 (± 7d) |

Patient #1: 1 X $10^5$ NK Cells/kg infused on day 42 or following ANC recover to at least 1000 cells/μL (whichever comes first).

If a patient is enrolled in a dose cohort but cell processing does not yield a sufficient number of cells to meet the cohort dose target, if the product otherwise fulfills the release criteria, the patient will be treated as planned but analyzed as additional data in the lower dose cohort according to the number of cells available. That patient will be replaced in the cohort enrollment numbers to ensure sufficient data for toxicity analysis.

### 3.1.1 NK Cell Infusion Definition of Dose Limiting Toxicity (DLT)

Dose-limiting toxicity is defined as follows:

- All grade 3 – 5 allergic reactions at infusion of NK-DLI
- Grade 4 neutropenia lasting > 28 days
- Grade 4 or 5 organ toxicity within 30 days of the last dose of NK-DLI, at least possibly related to the NK-DLI
- Steroid refractory Grade 3 or 4 acute GVHD within 45 days of the last dose of NK-DLI

### 3.1.2 Requirements for DLI-NK Cell Infusions

3.1.2.1 Requirements for the first DLI-NK infusion

- Equal to or < Grade 1 aGVHD
- Off corticosteroids for aGVHD treatment
- No uncontrolled infection is present
- No oxygen requirement is present
- No serious condition that in the opinion of the PI places the patient at increased risk from NK cell infusion

3.1.2.2 Requirements for second DLI-NK Cell Infusion

The second DLI-NK cell infusion can be given if the following criteria are met:

- Equal to or < Grade 1 aGVHD
- Off corticosteroids for aGVHD treatment
- ANC > 500
- Platelets > 20 K without transfusion support
- No uncontrolled infection is present
- No oxygen requirement is present
- Baseline or < Grade 3 ALT, AST and serum bilirubin.
- No serious condition that in the opinion of the PI places the patient at increased risk from NK cell infusion

The dose of the second DLI-NK cell infusion may be reduced to the next lower dose, at the PI's discretion if grade 1 GVHD is present. The infusion of the second DLI-NK may be delayed up to 100 days post-transplant to allow for resolution of any active issues that may have placed the patient at increased risk from NK cell infusion at the assigned time-point of the second infusion.

## 3.2 ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANT:

3.2.1 Donor Peripheral Blood Stem Cell Harvest:

3.2.1.1 Donor Mobilization and Collection of CD34+ PBSCs:

*Related Donor stem cell and NK cell mobilization with filgrastim*

After medical evaluation and clearance for suitability as an allogeneic donor, donors will undergo mobilization with filgrastim (G-CSF, Neupogen, Amgen). The first dose of filgrastim will be given in the clinic or doctor's office where the donor will be observed for approximately 90 minutes to rule out evidence of allergic reaction.

*Filgrastim administration:*

Minor donors will receive filgrastim as an outpatient at a dose of 10 µg/kg/day each morning by subcutaneous (SQ) injection beginning 4 days prior to scheduled collection. Adult donors will be dosed according to DTM "higher dose algorithm" as components will undergo further processing; and a high transplant cell dose is required (CD34 > 8 x$10^6$/kg). GSCF dosing instructions can be found in **Appendix 10**.

3.2.1.2 Donor stem cell collection by apheresis

For related donors, PBSC collection will be performed in the Dowling Apheresis Clinic of the Department of Transfusion Medicine (DTM). Bilateral peripheral venous access will be used

whenever possible. Alternatively, a temporary central venous catheter (CVC) will be placed for collection. If this is necessary, the donor will be admitted to the Clinical Center for CVC placement prior to starting apheresis. The CVC will be inserted by Critical Care or Interventional Radiology staff with the assistance of anesthesiology as indicated. The donor will remain hospitalized until collections are completed and the CVC is removed.

A DTM physician is within the immediate vicinity of the procedure or available within one minute by pager. Donors will receive divalent cation prophylaxis (Ca) to prevent citrate toxicity during apheresis, in accordance with standard DTM policies. The target (or "optimum") CD34 cell number harvested in the DTM Cell Processing Laboratory is $\geq$ 10 x $10^6$/kg, with a minimum of 8 x $10^6$/kg. The minimum post-selection CD34 dose that must be recovered in order to proceed with further therapy is 4 x $10^6$/kg. Since approximately 60% of the CD34 cells in the apheresis product are recovered following CD34 cell selection, the targeted CD34 dose to be collected by apheresis is >8 x .6 = 4.8 x $10^6$/kg recovered after CD34 selection of the apheresis component.

The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, based on peak CD34 cell mobilization response to filgrastim, optimum and minimum CD34 cell dose needed, and kilogram weight of recipient. Volume processed will range from 15 to 35 liters per procedure for 1 to 3 consecutive daily procedures, not to exceed a total of 75 liters over 3 days. In pediatric subjects, defined as less than 40 kg, a maximum of 5 total blood volumes will be processed per procedure, for up to 2-3 consecutive daily procedures. Collection will be conducted using the Cobe Spectra, or an equivalent instrument. This procedure typically takes 4 to 6 hours.

If $\geq$ 10 x $10^6$ $CD34^+$ cells per kg (recipient weight) are harvested after the first cycle of mobilization and apheresis, no further mobilization or collection will be performed, and the patient will be eligible to receive the stem cell transplant with that stem cell dose. In the event that < 10 x $10^6$ $CD34^+$ cells per kg are harvested after the first cycle of mobilization and apheresis, the donor may need to undergo additional mobilization and collection, depending on the final cell dose.

Unrelated donors will be collected according to similar procedures as described above, but at NMDP Centers in accordance with approved NMDP policies and procedures. Unrelated donor apheresis products will be transported immediately after donation to the NIH DTM Cell Processing Section for processing.

3.2.1.3 Stem Cell Product Processing

The PBSC from all donors' apheresis product will undergo targeted T-cell depletion by CD 34 positive selection (anti-CD34 beads) over a CliniMACS device (Miltenyi Biotec). Product will be adjusted post-selection for a cell count of 1 x $10^4$ CD3+ cells/kg, but will allow up to a maximum of 2 x $10^4$ CD3+ cells/kg for **unrelated donor** products. In recipients of **matched related donor** products, the CD3+ count will range from 1-2 x $10^5$ CD3+ cells/kg. Any additional CD3+ T-cells will be cryopreserved for potential future DLI. DLI will be stored in up to three aliquots at the following doses: 1 x $10^6$ CD3/kg; 1 x $10^7$ CD3/kg and 1 x $10^8$ CD3/kg, as feasible. If these doses cannot be attained, decisions regarding the appropriate dose and possible aliquots will be based on standard CPS, DTM procedures. PBSCs will typically be infused fresh,

but if needed, may be cryopreserved by methods currently in use and detailed in the Cell Processing Laboratory (CPS), DTM standard operating procedures, and stored in either the vapor or liquid phase of a liquid nitrogen storage tank according to standard CPS, DTM procedures.

The concentration of $CD34^+$ cells in the apheresis product will be determined by flow cytometry, and the number of $CD34^+$ cells in each bag will be calculated. For collections from related donors ONLY, where the pre-collection CD34 count predicts a yield of greater than $15 \times 10^6$ CD34+ cells/kg of recipient body weight, 10% of the total collection will be cryopreserved as separate aliquots and stored for research.

3.2.1.4 NK Cell Product Processing

NK Cells will be immunomagnetically selected from the $CD34^+$ cell depleted apheresis product using the CliniMacs device. Cells will be cryopreserved using standard techniques. Approximately 7-10 days prior to first infusion, the NK cell product will be thawed and placed in culture with irradiated KT64.4-1BBL artificial antigen presenting cells and rhIL15. Co-culture may be maintained for 8-10 days until the target dose is achieved according to standard operating procedures in DTM. If target cell dose is achieved, cells may be frozen and subsequent infusions given from the thawed product. The culture will be maintained in a closed system using bags. On the day of infusion, cells will be given fresh and infused into the patient as outlined in section **3.3.2.9**. Cryopreserved cells may be used as clinically indicated.

3.2.2 Biopsy of accessible tumor:

Where possible, biopsies will be obtained from adult patients with accessible tumor in order to establish tumor cell lines. Since the ability to generate immune responses against tumor cell lines from these patients represents an experimental question from which there is no benefit to individual patients, risks associated with acquisition of additional tumor tissue from patients with an established diagnosis must be minimized. If a biopsy is being obtained solely for experimental purposes, procedures used to obtain the additional tissue should be limited to fine needle aspiration, core biopsy, or open biopsy of readily accessible lesions. Patients should not be subjected to extensive surgeries such as thoracotomy or laparotomy. In addition, this procedure is entirely optional and the patient refusal of a biopsy will not prevent enrollment on this trial.

## 3.3 DRUG ADMINISTRATION AND TREATMENT MODIFICATIONS

3.3.1 Induction Chemotherapy

Unless the patient already has a functioning permanent CVC, one will be placed prior to initiation of chemotherapy. Patients may receive up to three cycles of induction chemotherapy regardless of CD4 count at the time of study entry.

The following tables are guidelines for schedules, rates and volumes, which, since these are standard induction chemotherapy regimens may vary slightly based on the clinical situation and guidance from personnel in the Pharmacy Department and clinical staff.

3.3.1.1 Induction Chemotherapy for lymphoma patients and some patients with leukemia:

EPOCH- Fludarabine may be administered as outpatient:

| Drug | Dose | Days |
|---|---|---|
| Fludarabine | 25 mg/m$^2$ per day IV<br>infusion over 30 minutes ± 10 minutes,<br>daily for 3 days | Days 1,2,3 |
| Etoposide | 50 mg/m$^2$ per day continuous IV<br>infusion over 24 hours ± 60 minutes, daily for 4 days | Days 1,2,3,4 |
| Doxorubicin | 10 mg/m$^2$ per day continuous IV<br>infusion over 24 hours ± 60 minutes, daily for 4 days | Days 1,2,3,4 |
| Vincristine | 0.4 mg/m$^2$ per day continuous IV<br>infusion over 24 hours ± 60 minutes, daily for 4 days | Days 1,2,3,4 |
| Cyclophosphamide | 750 mg/m$^2$ IV Infusion over 30 minutes ± 10 minutes x 1 dose | Day 5 |
| Prednisone | 60 mg/m$^2$ per day in 2-4 divided doses PO daily for 5 days | Days 1,2,3,4,5 |
| Filgrastim or pegfilgrastim | Filgrastim: 5 μg/kg per day SQ<br>Pegfilgrastim: 6 mg SQ, one dose. | Filgrastim: Daily from day 6 until ANC >1000/μl x 2 consecutive CBC's<br>Pegfilgrastim: for patients ≥ 40 kg, given 24-36 hours post chemotherapy (next cycle of chemo therapy must be ≥14 days). |

3.3.1.2 Induction chemotherapy for patients with leukemias (Non-myeloablative regimen):

| **Drug** | **Dose** | **Supportive Care** | **Days** |
|---|---|---|---|
| Fludarabine | 25 mg/m$^2$ per day IV infusion over 30 minutes ± 10 minutes, daily for 5 days. | IV pre-hydration 1 hour prior to fludarabine daily for 5 days with 0.9% NaCl and KCl 10 meq/liter at a rate of 90 ml/m$^2$/hour to a maximum rate of 100 ml/hour. Following fludarabine infusion, restart fluid at a rate of 90 ml/m$^2$/hour to a maximum rate of 100 ml/hour continue until cytarabine infusion begins. | Days 1,2,3,4,5 |
| Cytarabine* | 2000 mg/m$^2$ IV infusion over 4 hours ± 30 | a. Corticosteroid ophthalmic drops 2 drops to each eye every 6 hours starting prior to first dose and until 24 hours after the last dose of cytarabine completed. | Days 1,2,3,4,5 |

| | | | |
|---|---|---|---|
| | minutes, daily for 5 days. | b. Begin cytarabine doses 3.5 hours after completion of the preceding fludarabine.<br>b. Infuse in 250 ml of D5W.<br>c. IV post-hydration x 4 hours daily for 5 days with 0.9% NaCl and KCl 10 meq/liter at a rate of 90 ml/$m^2$/hour to a maximum rate of 100 ml/hour.<br>d. In the event of signs of CNS toxicity, cytarabine infusion will be interrupted and the M.D. notified. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine. | |
| Filgrastim | 5μg/kg per day SQ beginning the day PRIOR to initiation of chemotherapy. | | Daily from day 0 until ANC >1000/μl x 2 consecutive days |

3.3.1.3 **Subsequent Cycles**: Patients may receive subsequent cycles of chemotherapy as early as day 22 and up to 28 days after the previous cycle was initiated. Toxicities must resolve to < grade 2 in order to receive the next cycle with the following exceptions: liver function test elevations (if believed due to malignancy, manage as outlined in section **2.1.1.11**), neutropenia (ANC must be > 750/mm3 unless due to underlying disease in which case there is no grade restriction), lymphopenia, CD4 lymphopenia, leukopenia, and anemia (no grade restriction). An additional 14 days of recovery time is allowed before administration of subsequent cycles for resolution of toxicity or as medically indicated (e.g., documented infection).

3.3.1.4 Dose Modification for Cycle 2 and Cycle 3

1.) Dose Reduction: If the prior cycle of induction chemotherapy is associated with reversible grade 4 non-hematologic toxicity, dose reduction for that patient's subsequent cycles will be performed. In addition, if 2 patients experience the same grade 4 non-hematologic toxicity, dose reduction will be performed for all subsequent cycles for all patients. Dose reduction will be as follows:
   a) EPOCH-F: Etoposide, doxorubicin, and vincristine will be reduced from 4 days to 3 days of administration and prednisone will be reduced from 5 days to 4 days. In the event of this change, cyclophosphamide will be given on day 4 and filgrastim will be started on day 5. The doses of all medications will remain unchanged.
   b) FLAG: Fludarabine and cytarabine will be reduced from 5 days to 4 days of administration. The doses of all medications will remain unchanged.

3.3.1.5 Anti-emetics:

Routine anti-emetic prophylaxis and treatment should be employed. Corticosteroids may be used if clinically indicated.

3.3.1.6 Maximum dose of doxorubicin:

As long as it has been determined that patients have adequate cardiac function as outlined in section **2.1.1.9**, doxorubicin will be administered until a maximum cumulative total dose of 500 mg/m$^2$. Once this dose level has been reached, induction chemotherapy will not include doxorubicin. All other agents will be given according to the parameters outlined elsewhere in this section.

3.3.2 Transplantation:

On day 22 after the final cycle of induction chemotherapy, patients will be eligible to start pre-HSCT preparative regimen. However, transplant may be postponed for up to an additional 14 days in order to facilitate cell processing, patient recovery from illness or other unanticipated delays. The following clinical parameters must be met in order to proceed to the transplant portion of this protocol:

3.3.2.1 Clinical Parameters Required for BMT:

3.3.2.1.1 Non-hematologic toxicities of induction chemotherapy must resolve to < grade 2 in order to proceed. If more than 14 days of additional recovery time is necessary for resolution of toxicity or as medically indicated (e.g., documented infection), PI approval is required prior to proceeding to HSCT.

3.3.2.1.2 ECOG performance status of 0,1 or 2, or Karnofsy ≥ 60 or, for children ≤ 10 years of age, Lansky ≥ 60 (**Appendix 1**).

3.3.2.1.3 Life expectancy > 3 months.

3.3.2.1.4 Cardiac function: Left ventricular ejection fraction ≥45% by MUGA or ECHO, fractional shortening ≥28% by ECHO.

3.3.2.1.5 Renal function: age adjusted normal serum creatinine according to the table shown in 2.1.10 or a creatinine clearance of >60 ml/min/1.73 m$^2$.

3.3.2.1.6 Liver function: serum direct bilirubin <2 mg/dl, serum AST and ALT ≤ 2.5x upper limit of normal.

3.3.2.1.7 **Disease Status:** Patients must meet same disease criteria as enrollment as defined in section **2.1.1.1**

3.3.2.2 Inpatient care:

Patients will be admitted for peri-transplant care to NIH in accordance with CC and nursing policies. Patients will be hospitalized in single positive pressure isolation rooms.

3.3.2.3 Pre-HSCT Non- Myeloablative Preparative Regimen:

The following table is a guideline for schedules, rates and volumes, which, since these are standard pre-transplant chemotherapy regimens may vary slightly based on the clinical situation and guidance from personnel in the Pharmacy Department and clinical staff.

Pre-transplant chemotherapy will be administered as follows:

| **Drug** | **Dose** | **Days** |
|---|---|---|
| Fludarabine | 30 mg/m$^2$ per day IV infusion | Transplant Days –5, -4, -3, -2 |

| | | |
|---|---|---|
| | over 30 minutes ± 30 minutes, daily for 4 days | |
| Cyclophosphamide | 1200 mg/m$^2$ per day IV infusion<br>Over 2 hours ± 30 minutes, daily for 4 days | Transplant Days –5, -4, -3, -2 |
| Mesna* | 1200 mg/m$^2$ per day by continuous IV infusion, daily for 4 days. | Transplant Days –5, -4, -3, -2 |

*Mesna will be administered at a dose of 1200 mg/m$^2$ per day by continuous IV infusion on days –5, -4, -3, and –2. The first 100 mg/m$^2$ will be mixed with the cyclophosphamide in an appropriate amount of fluid based on the hydration rate. Immediately upon completion of this initial infusion, mesna at a dose of 1100 mg/m$^2$ will be infused over 22 hours until the next cyclophosphamide dose or until 22 hours after completion of the final dose of cyclophosphamide.

3.3.2.4 Pre-HSCT Myeloablative Preparative Regimen for AML or subjects who have received prior TBI, as clinically indicated:

The following table is a guideline for schedules, rates and volumes, which, since these are standard pre-transplant chemotherapy regimens may vary slightly based on the clinical situation and guidance from personnel in the Pharmacy Department and clinical staff.

| Day | Treatment |
|---|---|
| -6 | Anti Seizure Prophylaxis* |
| -5 to -2 | Fludarabine 40 mg/m$^2$/dose IV daily (1.3 mg/kg if <10kg)** |
| -5 to -2 | Busulfan 3.2 mg/kg/dose IV daily x 4 doses*** |
| -1 | Rest (may be extended an additional day if needed) |
| 0 | HSCT Infusion |

Note: Dose adjustments will be made for obesity, defined as > 125% of IBW:

Adjusted weight = 1.25*IBW (Please see **Appendix 13** for IBW and dose adjustment calculations.)

* Seizure prophylaxis is mandatory. The preferred regimen is lorazepam (0.02-0.05 mg/kg/dose, maximum dose: 2 mg) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. Alternative acceptable regimen is levetiracetam beginning 12 hours prior to busulfan and continue at least 24 hours after completion of busulfan. The dose of levetiracetam is 10 mg/kg/dose PO BID (maximum dose: 1000 mg). Loading dose and therapeutic monitoring are not necessary. For patients with a known seizure disorder, consider IV dosing with loading and therapeutic monitoring.

** ≥10 kg: Fludarabine 40 mg/m2 IV daily in 0.9% NaCl or D5W over 30 minutes on Days -5, -4, -3, -2 for a total of 4 doses.

< 10 kg: Fludarabine 1.33 mg/kg IV daily in 0.9% NaCl or D5W over 30 minutes on Days -5, -4, -3, -2 for a total of 4 doses.

*** Busulfan administered intravenously via a central venous catheter as a 3-hour infusion once daily for 4 consecutive days for a total of 4 doses. Doses will be adjusted to achieve an overall exposure target AUC of 900-1500 (micromole/liter)*minute.

3.3.2.5 Pre-HSCT Myeloablative Preparative Regimen for ALL:

The following table is a guideline for schedules, rates and volumes, which, since these are standard pre-transplant chemotherapy regimens may vary slightly based on the clinical situation and guidance from personnel in the Pharmacy Department and clinical staff. Prep should be continuous.

| Day | Treatment*, ** |
|---|---|
| -6 to -4 | TBI 200 cGy BID (see **Appendix 12** for TBI guidelines) |
| -3 to -2 | Cyclophosphamide 60 mg/kg/dose IV daily |
| -3 to -2 | MESNA 60 mg/kg/dose IV daily |
| -1 | Rest (may be extended an additional day if needed) |
| 0 | HSCT Infusion |

Note: Dose adjustments will be made for obesity, defined as > 125% of IBW:

Adjusted weight = 1.25*IBW (Please see **Appendix 13** for for IBW and dose adjustment calculations.)

*The order of the cyclophosphamide/TBI may be switched to accommodate scheduling needs

**A CNS boost, if indicated, will precede the start of the conditioning regimen.

Mesna infusion should start simultaneously with the cyclophosphamide and be administered by continuous IV infusion on days -3 and -2. The Mesna infusion should continue for a minimum of 22 – 24 hours after the last dose of cyclophosphamide. The entire Mesna dose prescribed should be administered each day of cyclophosphamide therapy.

3.3.2.6 Hydration During Pre-HSCT Preparative Chemotherapy:

IV hydration will be initiated 12 hours prior to cyclophosphamide (on day –7 of the non myeloablative transplant, day -4 of the myeloablative ALL transplant) using 0.45% NaCl with 5% dextrose supplemented with KCl 10 meq/liter at a rate of 100 ml/$m^2$/hour continuing until 24 hours after the last cyclophosphamide dose has been completed. During hydration, IV furosemide (0.5 mg/kg/dose to a maximum of 20 mg) may be administered as needed to maintain normal urine output and fluid balance. During hydration, serum electrolyte levels will be monitored every 12 hours and IV fluid content and KCl supplementation will be adjusted to maintain normal serum electrolyte levels.

Hydration volume, composition and/or rate may be modified based on patient condition and investigator assessment, but may not go below the minimum of 65 ml/$m^2$/hour.

3.3.2.7 GVHD Prophylaxis with calcineurin inhibitor:

- Tacrolimus will be initiated on the day -1 (at least 24 hours before the stem cell infusion) typically starting at a dose of 0.02 - 0.03 mg/kg/day as a continuous infusion. Once oral feedings are tolerated and there is resolution of mucositis, parenteral tacrolimus will be discontinued. Twelve hours later, oral tacrolimus will be initiated at a dose of 0.1-0.15 mg/kg/day in two divided doses every 12 hours.
- The tacrolimus dose will be titrated to maintain a trough level of 5-10 ng/ml. Tacrolimus levels should NOT be drawn from the CVL lumen used for tacrolimus infusion.

OR

- Cyclosporine-A will be initiated on the day before the transplant (day –1) starting at a dose of 2 mg/kg/dose IV over 2-hours q 12 hours.
- The dose will be titrated to maintain an approximately trough level of 200-250 ng/ml (monoclonal RIA methodology). CSA levels should not be drawn from the CVL lumen used for CSA infusion.
- Once the patient is able to tolerate oral feedings, CSA will be converted to an equivalent oral dose. The starting oral dose should be approximately 2-times the IV dose given in a q 12 hour schedule. The dose should be titrated to a trough level of 200-250 ng/ml (monoclonal RIA methodology).

- Tacrolimus is preferred over cyclosporine-A, however if tacrolimus is unavailable, or the laboratory reagents needed to monitor tacrolimus serum levels are unavailable, cyclosporine-A may be substituted,
- Calcineurin inhibitor will continue until day 100 post-transplant, at which point it will be gradually tapered as long as the level of acute-GVHD is < grade 2 and there is no chronic-GVHD. Taper will consist of a 5 to 10% dose reduction each week and will be discontinued by around day 180 post-transplant.
- The decision to taper Caclineurin inhibitor before day 100 will only be permitted if clinically indicated and after PI approval. Specifically, taper before day 100 may be permitted for the treatment of progressive disease and/or low levels of donor chimerism.

3.3.2.8 Anti-emetics:

Routine anti-emetic prophylaxis and treatment should be employed. Corticosteroids may be used if clinically indicated.

3.3.2.9 Stem Cell Infusion:

On day 0, the patient will receive fresh donor CD34+ PBSCs via intravenous infusion. (Cryopreserved cells may also be used in clinically indicated.)

If the stem cells are cryopreserved, the product will be thawed and administered IV immediately at a rate of approximately 10-15 ml/min or as tolerated based on volume status and/or DMSO toxicity. Do not exceed 20 ml of product per kg body weight (if 10% DMSO) or 40 ml of product per kg body weight (if 5% DMSO) so that per 24-hour period so that total DMSO administered is less that 1 mL/kg/day.

The target dose of the PBSC is ≥ 5 x $10^6$ $CD34^+$ cells per kg recipient body weight.

a. Pre-medications: Patients will receive the following medications approximately 30-60 minutes prior to stem cell infusion:
   i. Diphenhydramine 0.5 - 1 mg/kg/dose (maximum 50 mg/dose) IV
   ii. Acetaminophen 15 mg/kg/dose (maximum 650 mg/dose) PO
   iii. If the patient has been premedicated earlier in the day for blood product or other infusion, pre-medication is not warranted if given within 4 hours of stem cell infusion.
b. Hydration During Stem Cell Infusion: IV hydration will be initiated at a minimum of 12 hours prior to stem cell infusion (on day –1) using 0.9% NaCl supplemented with KCl 10 meq/liter. Patients will receive hydration at a rate of 90 ml/$m^2$/hour to a maximum rate of 100 ml/hour. Hydration will continue at a minimum of 12 hours after the stem cell infusion has been completed.
c. Monitoring During Stem Cell Infusion (Inclusive of T-cell Addback as a separate infusion): General monitoring guidelines will include vital signs and oxygen saturation q 15 minutes (+/- 10 minutes) until one hour after completion of infusion (s) and will follow NIH Clnical Center Nursing and Patient Care Services Procedure "Administration of products for Cellular Therapy." (http://intranet.cc.nih.gov/nursing/practicedocs/procedures_pdf/PRO_Cellular_Therapy_Infusion.pdf)
d. Supplemental oxygen will be available at the bedside.
e. If an allergic or other acute reaction occurs, studies appropriate for investigation of a transfusion reaction will be performed (urinalysis, CBC, Coomb's test).

3.3.2.10 Growth Factor Administration Post-transplant:

After stem cell infusion on day 0, filgrastim will be started at a dose of 5µg/kg/day SQ, unless contraindicated. Filgrastim will continue until the ANC is greater than 5000 cells/µl (post nadir) on two consecutive blood draws. If the patient has impaired renal function, pegfilgrastim may be administered in place of filgrastim at 6 mg (one time dose) for patients ≥ 40 kg, on day +1.

3.3.2.11 NK Cell Infusion:

On day 21 (+/- 3 days) after stem cell infusion, the dose of NK cells will be infused intravenously over approximately 10 -20 minutes. In the absence of DLT, the NK cell infusion will be repeated 28 (+/- 7 days) days following the first infusion for a total of 2 infusions, as per the dose descriptions in Section **3.1**. Any adverse reaction should prompt interruption of the infusion and should be reported to the principal investigator.

Patients will receive the following medications approximately 30-60 minutes prior to NK cell infusion:

1.) Diphenhydramine 0.5 - 1 mg/kg/dose (maximum 50 mg/dose) IV;
2.) Acetaminophen 15 mg/kg/dose (maximum 650 mg/dose) PO
3.) If the patient has been premedicated earlier in the day for blood product or other infusion, pre-medication is not warranted if given within 4 hours of stem cell infusion

Safety monitoring during and after infusion will follow NIH clnical center guidelines (http://intranet.cc.nih.gov/nursing/practicedocs/procedures_pdf/PRO_Cellular_Therapy_Infusion.pdf) and will include vital signs (prior to infusion, every 15 minutes (+/- 10 minutes) for 1 hour

after the start of infusion, every 30 minutes (+/- 15 minutes) for the second hour and then hourly until stable.

An NK-DLI infusion may be delayed in patients with significant complications (eg. severe infection, engraftment syndrome, GVHD flare) that according to the protocol PI or AI, may be in the best interest of the patient, and later administered when the patient has sufficiently recovered. Cells must meet the same release criteria as the originally scheduled dose regimen. For statistical analysis, patients receiving infusions outside of the planned ± 3 day or 7 day time frame will be analyzed separately and reported descriptively.

NK cells may remain in culture for up to an additional 7-14 days. If both doses of NK cells can be delivered within the prescribed time, this will be considered feasible delivery of NK-DLI.

If a DLT occurs, no further NK cells will be given to that patient.

### 3.4 ON STUDY PROTOCOL EVALUATION (APPENDIX 2 A):

3.4.1 Baseline

3.4.1.1 Spinal fluid for cell count and cytology (for patients with acute leukemia or NHL).

3.4.1.2 PCR of mini-satellite regions (STR profile) for future determination of chimerism (donor and patient). These studies may be performed at any time prior to entry without time limitation.

3.4.1.3 Antibody screen for varicella, HSV, and toxoplasma (patient only) and CMV, EBV (patient and donor).

3.4.1.4 PPD with appropriate control (for patients considered to be at high risk).

3.4.1.5 CBC with differential (patient and donor). Must be repeated within 72 hours of first collection and within 24 hours of subsequent collections (donor).

3.4.1.6 TBNK (patient only)

3.4.1.7 Urinalysis, (patient and donor).

3.4.1.8 Lupus anti-coagulant (patient only), as indicated.

3.4.1.9 Serum iron, ferritin and transferrin (patient only).

3.4.1.10 24-hour urine for creatinine clearance, if indicated.

3.4.1.11 Spot urine for calcium/creatinine ratio (patient only).

3.4.1.12 Radiologic studies that measure identifiable disease may be repeated prior to each cycle of immune depleting chemotherapy and prior to the conditioning regimen, at the PI's discretion.

3.4.2 Induction Chemotherapy Cycles (EPOCH-F or FLAG cycles)

3.4.2.1 H&P prior to each cycle and weekly during induction.

3.4.2.2 CBC, differential, platelets and prior to each cycle and two times per week during induction.

3.4.2.3 Laboratory evaluations: *Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid,*- prior to each cycle and one time per week during induction.

3.4.2.4 Urinalysis prior to each cycle.

3.4.2.5 Urine or Serum Pregnancy test prior to each cycle, for all females of childbearing potential.

3.4.2.6 Disease staging evaluation - as clinically indicated based on disease and sites of disease (see section **2.2**).

3.4.2.7 TBNK or NK panel (CD4 count) within 7 days prior to each cycle.

3.4.3 Pre-HSCT evaluation

3.4.3.1 H&P

3.4.3.2 CBC, differential, platelets

3.4.3.3 Laboratory evaluations: *Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid*

3.4.3.4 Urinalysis

3.4.3.5 Complete disease staging evaluation – staging based on disease and sites of disease within 17 days prior to start of transplant preparative regimen.

3.4.3.6 EKG and MUGA or ECHO

3.4.3.7 Research studies (Section **3.5**, **Appendix 4**) (When ALC > 200 K/uL ONLY)

a. Lymphocyte Phenotyping:
- TBNK (CD4 count) or
- NK Subset (except on Fridays) within 7 days prior to each cycle (See **Appendix 4**).

3.4.4 HSCT Hospitalization

3.4.4.1 H&P daily

a. Twice daily weights (from transplant through date of engraftment)
b. DAT daily on days 6-10 (for patients with ABO histo-incompatabilities ONLY)

3.4.4.2 CBC, differential, platelets daily.

3.4.4.3 Laboratory evaluations: *Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT,*

*AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid* daily.

3.4.4.4 Type and screen every 4 days (as clinically indicated)

3.4.4.5 Reticulocyte count, haptoglobin, IGG level, EBV, CMV, HHV6, adenovirus monitoring weekly (during hospitalization).

3.4.4.6 Urinalysis daily

3.4.4.7 Research studies (Section **3.5**, **Appendix 4**)

3.4.4.8 Disease staging evaluation (as clinically indicated based on disease sites)

3.4.5 Evaluations After HSCT discharge through day 100 (±30 days), as clinically indicated

3.4.5.1 H&P twice weekly

3.4.5.2 CBC, differential, platelets weekly.

3.4.5.3 EBV, CMV, HHV6, adenovirus monitoring weekly.

3.4.5.4 Type and screen and IGG level, weekly as clinically indicated.

3.4.5.5 Laboratory evaluations: *Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid* weekly.

3.4.5.6 Iron studies - serum iron, transferrin, ferritin, reticulocyte count, monthly as clinically indicated.

3.4.5.7 Bone marrow biopsy and aspirate day 28 (as indicated).

3.4.5.8 Research studies (Section **3.5**, **Appendix 4**).

3.4.5.9 Blood for bcr-abl PCR 28-35 days post final NK cell infusion.

3.4.6 Evaluations after Day 100 (**Appendix 2**) (±30 - 90 days):

Disease evaluations may occur before Day 100 or at any time after day 100 based on signs and symptoms or PI's suspicion of disease recurrence. Follow-up visits will be clinically determined after day 100, but at least once monthly. NIH scheduled visits will occur around day 100, day 150, day 180, day 270, and day 365 post-transplant. These timepoints are used a guidelines but may be altered based on each patient's clinical condition. Patients will then be followed approximately every 3 months for the second post-transplant year, and then every 6 months for the third post-transplant year, and then yearly until at least 3 years post-transplant. Evaluations will include the following as clinically indicated:

3.4.6.1 H&P including symptoms of GVHD (see Appendices 6-8), infection, and respiratory dysfunction.

3.4.6.2 CBC and reticulocyte count

3.4.6.3 Laboratory evaluations: *Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total,*

*Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid*

3.4.6.4 IgG levels monthly until IVIG supplementation discontinued.

3.4.6.5 Blood type, red cell phenotype, and ABO isohemagglutinin titers (in cases of ABO incompatibility only) as clinically indicated.

3.4.6.6 Antibody titers to previous vaccinations or infections (e.g., diphtheria, HIB, HBSAb, pneumococcus, tetanus, varicella) at or beyond 12 months post-HSCT and off IVIG for at least 3 months.

3.4.6.7 Disease staging evaluation (as clinically indicated based on disease sites): All studies that identify disease will be performed around day 100, every 3 months for the first year, every 6 months for the second and third year, and yearly for years 4-5, or as clinically indicated.

3.4.6.8 Organ function evaluation:

a. Pulmonary function tests with DLCO q 3 months if GVHD, otherwise every 6 – 12 months and as clinically indicated.

b. Cardiac tests: EKG and MUGA or ECHO at around 6 months, 1 year, and then yearly until year 5 ± 3-4 months.

c. Endocrine studies as clinically indicated.

3.4.6.9 Research studies (Section **3.5**, **Appendix 4**) including KIR/HLA genotyping/immunogenetics at or around Day 100±30 days.

3.4.7 Related Donor Evaluations During Treatment (**Appendix 2 B**)

3.4.7.1 Donors will have an interim health assessment performed by DTM staff immediately before each collection procedure.

3.4.7.2 Infectious disease testing will be performed no more than 30 days prior to each collection.

3.4.7.3 If a CVC is required, CBC, PT, PTT, and history and physical must be performed within 72 hours prior to placement.

3.4.7.4 Donors will have a physical examination, including a CBC, in the ETIB or POB clinic or by their primary physician within one week of each collection.

## 3.5 NCI/NIH BIOLOGIC STUDIES

Blood sample volume for the research purposes of this study will be restricted. The amount of blood drawn from adults (those 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, in an 8 week period. The amount of blood drawn for research purposes from pediatric patient subjects (those under 18 years of age) will not exceed 5 mL/kg in a single day, and no more than 9.5 mL/kg over an 8 week period.

In the event that blood draws are limited due to these restrictions, research studies will be performed in order of priority as listed below and in **Appendix 4**.

3.5.1 Proposal for Immune Function Studies (Brown)

3.5.1.1 Background: to evaluate the effects of the chemotherapy regimen on host immune depletion/reconstitution.

3.5.1.2 Rationale: Analysis will consist of flow cytometry to detect depletion of lymphoid sub-populations during therapy.

3.5.1.3 Objectives / Specific Aims:

3.5.1.3.1 Immunophenotyping

Blood samples (5ml lavender top tube) will be drawn

Samples should be delivered to the Clinical Pathology Flow Cytometry Laboratory, Attn. Margaret Brown, Building 10, Room 2C410, 301-496-4879. Test must be scheduled prior to sending.

Time points for Lymphocyte Phenotyping–NK Subset samples will be: 1) On-study; post-HSCT at 24 hours (+/- 24 hours) post NK#1 and #2 infusion (+/- 48 hours) and on day 28 (+/- 7), day 60 (+/- 21), and day 100 (+/- 30), and then every 3 months (+/- 1 mo) for the first two years post-transplant. Samples will not be drawn if ALC is < 200 K/uL.

Time points for Lymphocyte Phenotyping TBNK samples will be: 1) Prior to cycle 2 and 3 of induction chemotherapy (within 7 days of each cycle); 2) Prior to pre-HSCT preparative chemotherapy, (day –7), and 3) Prior to HSCT (day 0) if ALC is > 200 K/uL. Samples will not be drawn if ALC is < 200 K/uLSamples should be delivered to the Clinical Pathology Flow Cytometry Laboratory, Attn. Margaret Brown, Building 10, Room 2C410, 301-496-4879.

3.5.2 Chimerism Analysis (Kurlander)

Blood samples (10cc and, if doing cell sorting, an additional 20* cc in yellow top ACD tubes) will be drawn to evaluate the relative percentage of donor and host chimerism post-transplant.

Samples should be delivered to the clinical laboratory of Dr. Roger Kurlander in Building 10, hematology laboratory (301-435-6399)

* Cell sorting will be done on day 14 and repeated on day 28 if mixed chimerism (<98% donor) is found on day 14.

Timepoints for samples will be: 1) Eligibility; 2) Day 14 (+/- 5), day 28(+/- 7), day 60(+/- 21), day 100(+/- 30) ; and 2) After day 100 as clinically indicated.

3.5.3 Serum Cytokine Analysis (Lab)

Blood samples (5 cc in red-top serum collection tubes) will be drawn to evaluate circulating cytokine levels (including IL7 and IL15) and possible correlation with the degree of CD4 lymphopenia, immune reconstitution, engraftment, and clinical complications such as graft versus host disease and infections. The serum will be separated and frozen for subsequent analysis by high sensitivity colorimetric enzyme linked immunosorbant assay.

Timepoints for samples will be: 1) On-Study; 2) Prior to pre-HSCT preparative chemotherapy (day –7); 3) Prior to HSCT (day 0); and 4) day prior to or morning of each NK infusion (before infusion) then day 1, 2, 7 (+/- 48 hr) and 14 (+/- 48 hr) days post each NK infusion, then days 60 (+/- 21) and 100(+/- 30), and then every 3 months (+/- 1 mo) for the first two years post-transplant.

Samples should be picked up by courier (301-846-5893) and delivered to Frederick Repository.

3.5.4 TH1/TH2 Cytokine Profile (Frederick Repository)

Blood samples (1 cc in red-top serum collection tubes) will be drawn to evaluate the types of cytokines that are present in the allogeneic environment, and correlate their levels with occurrence of potential GVHD and infectious complications. Blood samples will be analyzed by multiplex cytokine bead array technology.

Timepoints for samples will be: 1) On-Study; 2) Prior to pre-HSCT preparative chemotherapy (day –7); 3) Prior to HSCT (day 0); and 4) and post-HSCT on day 1, day prior to or morning of each NK infusion (before infusion) then day 1, 2, 7 (+/- 48 hr) and 14 days (+/- 48 hr) post each NK infusion, then days 60 (+/- 21) and 100 (+/- 30), and then every 3 months (+/- 1 mo) for the first two years post-transplant.

Samples should be picked up by courier (301-846-5893) and delivered to Frederick Repository.

3.5.5 KIR/HLA genotyping/immunogenetics (Carolyn Hurley – Georgetown Univ.) complete

3.5.5.1 Objective: Retrospectively evaluate for the presence or absence of specific KIR genes to determine if the presence of a specific KIR in the absence of its HLA ligand (ie KIR "mismatch") correlates with enhanced engraftment, increased anti-tumor activity and less infectious complications.

3.5.5.2 Rationale: Patients receiving KIR mismatched NK-DLI may have better NK activity due to the absence of inhibitory signals, but it is not clear if KIR mismatch is needed in the setting of multiple activating signals provided during the NK cell expansion process.

3.5.5.3 Methods: Presence or absence of specific KIR genes will be determined by locus specific PCR amplification followed by gel electrophoresis. The panel of primer pairs will detect KIR2DL1-2DL5, 2DS1-2DS5, 3DL1-3DL3, 3DS1 and two pseudogenes. Followup sequencing of KIR alleles may be performed if required to identify expressed alleles. Timepoint for samples will be prior to SCT (day 0) and day 100. Samples (1-2 ml blood in ACD or 3 buccal swabs will be kept at room temperature and will picked up by the research nurse. They will then be shipped deidentified to Lihua Hou / Carolyn Hurley at C. W. Bill Young Marrow Program/Georgetown University, 3rd Floor, 11333 Woodglen Drive, Rockville, MD 20852, 301-998-8900.

3.5.6 Viral monitoring for functional Immune Status

3.5.6.1 Objective: Serially monitor viral reactivation in transplant patients. Compare viral reactivation to historic controls on protocol 02-C-0259 and 01-C-0125. Monitor for a meaningful correlation between circulating NK cell levels and viral reactivation.

3.5.6.2 Rationale: Patients receiving TCD grafts carry a higher risk for viral infections and reactivation. Viral reactivation is a marker for T-cell immune dysregulation. In particular, HHV6 is a marker of cell mediated viral response and a marker of inflammation.

3.5.6.3 Methods: Weekly PCR surveillance of EBV, CMV, HHV6, and adenovirus through the Clinical Center central laboratory, Section of Microbiology.

3.5.7 Research Testing of Biopsy Samples

3.5.7.1 Xenograft Models: Human tumors can be transferred to immunodeficient mice. These xenografts models can be used to study tumor biology and treatment. Samples from consenting adult patients with solid tumor malignancies will be used for pre-clinical testing and development of immunotherapy strategies. Recent studies exemplify the important role for xenograft models in the pre-clinical development of IGFR-1 antibodies. Informed consent will be required for this purpose, and subjects will be given the option to decline having xenograft models established from their samples.

Sample requirements: Fresh tumor samples in DMEN + 10% FBS, (optimally 0.5 cm in one dimension). Samples will be minced into 1mm fragments and injected into immunodeficient mice in a location appropriate for specific tumor growth (adrenal gland for neuroblastoma, bone for osteosarcoma). Must schedule with lab in advance.

3.5.7.2 Cell line establishment: Attempts will be made to establish solid tumor cell lines lines with the aim to identify potential molecular targets for new therapy development. Cell lines may also be used to perform in vitro testing of new agents. Informed consent will be required for this purpose, and subjects will be given the option to decline having cell lines established from their samples.

Sample requirements: Fresh tumor samples in DMEN + 10% FBS, (optimally 0.5 cm in one dimension). Samples will be minced into 1mm fragments and cells will be placed in flasks with complete growth media and incubated in humidified tissue culture incubator.

3.5.8 NIH Policy on Research Use of Stored Human Samples, Specimens, or Data:

Blood and tissue collected during the course of this study will follow NIH guidelines for the research use of human samples. Most samples will initially be sent to the Central Repository for NCI/Frederick using established procedures. All samples will be stored in monitored freezers/refrigerators either in the Central Repository for NCI/Frederick or in the investigator's laboratory at specified temperatures with alarm systems in place. All samples will be tracked by unique sample identifiers. A secure computer database system will be used to track all samples collected on this protocol; the system will contain data that includes but is not limited to the unique sample identifiers, storage locations and conditions, biologic study results, information about the sample origins (as appropriate), and corresponding records of all derivatives generated from samples collected on this protocol. The system will employ mechanisms for restricting users to viewing only the level of data appropriate for each individual user, will provide the capability to audit any data modification, and will be maintained and backed up according to established standards.

Specimens will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland. Any new uses of human subject samples collected during the course of this trial must be reviewed and approved by the NCI IRB. Any loss or unintentional destruction of the samples will be reported to the IRB.

## 3.6 CONCURRENT THERAPY

3.6.1 Treatment of Graft-Versus-Host Disease:

In the event a patient develops GVHD, the subsequent procedures will be followed. Standard clinical and/or biopsy criteria will be used to establish the diagnosis of GVHD, attempts will be made to obtain histiologic confirmation. Acute GVHD (aGVHD) will be graded by the CIBMTR

criteria (**Appendix 5**) and Chronic GVHD (cGVHD) by the NIH cGVHD Consortium Consensus Criteria (**Appendix 6** and **Appendix 7**). Patients will be monitored for GVHD over the entire 5-year post-HSCT observational period. Patients with grade 1 acute GVHD will not receive systemic therapy. In general, patients with ≥ grade 2 acute GVHD will be treated with corticosteroids. Patients who fail to respond satisfactorily to corticosteroids will be considered for other standard anti-GVHD therapies, and/or investigational GVHD treatment protocols if available. Simiarly, patients with cGVHD will be treated with corticosteroids and/or calcineurin inhibitors. Patients will also be considered for other standard anti-GVHD therapies, and/or investigational GVHD treatment protocols if available. Established guidelines, which are regularly reviewed and updated for the treatment of GVHD as outlined by the NIH BMT consortium will be followed. These guidelines can be found at http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml.

3.6.2 Immunosuppressants

Calcineurin inhibitor as described in section **3.3.2.6**.

3.6.3 Infection Prophylaxis

Infectious prophylaxis as outlined in section **4.1**.

3.6.4 GI Protective Agents

Patients on corticosteroids must be on H2 blockade or other gastrointestinal protective agents.

3.6.5 Hematologic and Blood Product Support

3.6.5.1 Platelet transfusion: In attempt to prevent bleeding, platelet counts should be maintained > 20,000/ul (or as clinically indicated) and >50,000 prior to apheresis. Single donor platelets should be employed whenever possible in attempt to decrease donor exposure and the risk of alloimmunization.

3.6.5.2 Red blood cell transfusion: Packed RBCs should be used to maintain a hemoglobin > 8 gm/dL for ABO compatible transplants and >10 gm/dL in ABO minor mismatch transplants (or as clinically indicated).

3.6.5.3 Irradiation: To reduce the risk of transfusion associated graft-vs-host disease, all cellular blood products must be irradiated.

3.6.5.4 Leukodepletion: To decrease the risk of febrile reactions and alloimmunization, platelets and red cells should be leukoreduced.

3.6.6 Concurrent Therapy for Extramedullary Leukemia or CNS Lymphoma:

Concurrent therapy or prophylaxis for testicular leukemia, CNS leukemia, and CNS lymphoma including standard intrathecal chemotherapy and/or radiation therapy will be allowed as clinically indicated after approval by the PI. Such treatment may continue until the planned course is completed. Patients must be in CNS remission at the time of protocol enrollment.

3.6.6.1 CNS Prophylaxis for Patients with ALL: Patients with ALL may receive intrathecal (IT) methotrexate for 5 doses after transplant.

a. Dose**:** By age as follows:

| Age (years) | Methotrexate (mg)* | Volume (ml) |
|---|---|---|
| 3-8 | 12 | 8 |
| ≥9 | 15 | 10 |

b. Administration: To facilitate CNS delivery, the volume of CSF removed should be approximate the volume administered and patients should remain prone for 30 minutes after IT administration.

c. Schedule: Approximately weekly x 5 doses to begin after myeloid recovery, not before post-transplant day 28.

d. Antiemesis: Routine (e.g., ondansetron) should be considered prior to each dose of methotrexate in patients with a history of nausea associated with intrathecal chemotherapy.

*Methotrexate may be substituted with hydrocortisone/cytarabine, or Triple IT (MTX/AraC/HC) may be given, if clinically indicated, and dosing will be performed as per standard Children's Oncology Group (COG) doses (example below):

| Age (years) | Cytarabine (mg) | Hydrocortisone (mg) |
|---|---|---|
| 3-8 | 24 | 12 |
| ≥9 | 30 | 15 |

3.6.7 Treatment of Progressive Disease Post-transplant:

Patients who have progressive disease after day +100 post-transplant may receive donor lymphocyte infusions (DLI) since this has been effective in the management of post-BMT relapse. Patients who have been started on immunosuppression for GVHD treatment will undergo sequential withdrawal of immunosuppression prior to DLI. As long as there is no evidence for active GVHD, patients may be eligible to receive unmodified DLI. If deemed necessary to combat rapidly progressive disease, adjunctive chemotherapy using standard disease-specific agents may be employed in conjunction with this approach with PI approval. The specific chemotherapy to be used should be decided by the patient's oncology and BMT teams in consultation with the PI. Lympholytic agents (e.g., corticosteroids) should be avoided. Alternatively, in the event of progressive disease after day +100, the patient may be taken off-study and referred for conventional therapy or participation on other NCI investigational trials for which they might be eligible.

3.6.7.1 **LEUKAPHERESIS PROCEDURE TO COLLECT CELLS FOR DLI**: Donation of DLI shall be by standard lymphapheresis, during which 2 to 5 blood volumes will be processed as estimated by recipient weight and target cell harvest dose (maximum 5 total blood volumes) in children (defined as less than 40 kg), or 7 to 10 liters of blood in adults will be processed, depending on recipient weight (volume processed to be determined by

DTM staff in consultation with PI). Non-mobilized donor lymphocytes will be collected by apheresis and processed as per standard NIH DTM procedures. The lymphapheresis will undergo red cell or plasma depletion for major or minor ABO incompatibility, respectively, according to CPS, DTM policies and procedures. In the event that some or all of the donor lymphocytes will be administered in the future, the lymphapheresis product will be cryopreserved and stored according to CPS, DTM standard operating procedures. 10% of the total lymphapheresis product of related donor ONLY will be cryopreserved as a separate aliquot and stored for research. Use of cryopreserved DLI collected as part of the original PBSCT collection may be used if clinically indicated.

3.6.7.2 **DLI ADMINISTRATION**: DLI will generally be administered at a starting dose of 1 x $10^6$ $CD3^+$ T-cells/kg recipient body weight by IV infusion. A higher starting may be used as clinicaly indicated. Additional DLI may be administered in escalating doses in increments of 0.5-1 $\log_{10}$ to a maximum dose of 1 x $10^8$ $CD3^+$ T-cells/kg every 2-4 weeks provided there has been no improvement in malignant disease and there remains no evidence of GVHD.

Patients will receive the following medications approximately 30-60 minutes prior to DLI cell infusion:

1.) Diphenhydramine 0.5 - 1 mg/kg/dose (maximum 50 mg/dose) IV or PO over 10-15 minutes;

2.) Acetaminophen 15 mg/kg/dose (maximum 650 mg/dose) PO

3.) If the patient has been premedicated earlier in the day for blood product or other infusion, pre-medication is not warranted if given within 4 hours of stem cell infusion

Safety monitoring during and after infusion will include vital signs (blood pressure, heart rate, respiratory rate) prior to infusion, and 15 minutes post infusion and more frequently if clinically indicated.

3.6.8 Treatment of Mixed Chimerism Post-transplant:

If a patient has ≤ 95% donor lymphoid chimerism on day +28 post-transplant, repeat analysis will be performed until complete chimerism is achieved or a pattern of stable mixed chimerism or graft rejection is established. For patients with significant reductions in donor chimerism as determined by the PI, DLI (as per Section **3.6.7**) will be considered. If patient is on immunosuppression for GVHD) sequential withdrawal of immunosuppression will precede DLI administration. This approach has been successfully employed in pediatric patients with ALL, AML, and MDS who had progressive mixed chimerism after conventional myeloablative allogeneic BMT(212). If at any time a patient has < 5% donor lymphoid chimerism, they will be taken off treatment and consideration will be given for conventional HSCT.

3.6.9 Treatment of Graft Failure/Graft Rejection:

If a patient develops graft rejection (as evident by decreasing hematopoiesis and decreasing CD3 donor chimerism) or has graft failure (lack of engraftment) they will be taken off treatment and consideration will be given for conventional HSCT on-study. Therapy for the

treatment of graft rejection will be determined as clinically indicated but may include serotherapy such as anti-thymocyte globulin, alemtuzumab and/or second transplant.

## 3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.7.1 Criteria for removal from protocol therapy

- Unacceptable toxicity as defined in section **3.1.1**.
- Inability to proceed with transplantation (failure to meet criteria).
- Progressive disease during induction therapy.
- Irreversible non-hematologic grade 4 toxicity during induction chemotherapy. Any other non-hematologic toxicity during induction chemotherapy of sufficient severity or duration that in the opinion of the PI poses prohibitive risks to further chemotherapy.
- Progressive disease post tranplant, which requires anti-neoplastic therapy.
- Patients unable to comply with study requirements will come off treatment.
- < 5% donor lymphoid chimerism at or beyond day +28, or graft rejection/failure
- Enrollment on another clinical trial for treatment of progressive disease or graft rejection.
- Recipients will come off treatment upon completion of study follow-up period (5 year post PBSCT). These patients with be followed for survival and development of chronic GVHD.

3.7.2 Off-Study Criteria

- Recipient death.
- Recipient withdrawal of consent
- Lost to follow-up
- Investigator discretion
- Completed study follow-up period
- Donors will come off-study at the time of recipient off-study date

Note: all minors who sign the treatment consent will be kept on study and followed until the age of 18 to allow for consent.

3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

## 3.8 END OF STUDY EVALUATION (APPENDIX 2)

The following tests and procedures should be performed, if possible, at the time a patient comes off study regardless of the reason for coming off study, unless the test or procedure has been performed in the last 4 weeks (2 weeks for physical examination and laboratory assessment).

3.8.1 History and physical examination.

3.8.2 Laboratory Assessment: Iron studies (serum iron, ferritin, and transferrin), Complete blood count, differential and platelet count. Chem 20 and urinalysis.

3.8.3 Disease restaging

3.8.4 Apheresis. (Optional)

### 3.9 DURATION OF STUDY

Patients will be followed annually for up to year 3 after completing treatment on study, and annually by phone for survival status.

## 4 SUPPORTIVE CARE

### 4.1 INFECTION PROPHYLAXIS

Routine measures to prevent infection in accordance with standard of care practice. For patients enrolled at the NIH, the recommendations following the NIH Allogeneic Stem Cell Transplantation guidelines should be employed (http://intranet.cc.nih.gov/bmt/education/infectious-mgmt-guidelines.shtml).

### 4.2 MUCOSITIS PROPHYLAXIS

Kepivance™ (palifermin), a human keratinocyte growth factor (KGF), may be administered to reduce the incidence or severity of mucositis. Palifermin will be administered at a dose of 60 mcg/kg/day IV X 3 doses, given twice. The first 3 daily doses should be administered prior to the preparative chemotherapy with the 3rd dose completed at least 24 hours prior to starting the preparative regimen. The last 3 daily doses should be administered starting on day +1.

## 5 DATA COLLECTION AND EVALUATION

### 5.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

Donor AE data will be collected through 1 week following collection.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.

- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Recipient and Donor data will also be reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) registry as required by law. Recipients and donors will be offered the opportunity to consent to the use of this information, and additional baseline and outcome data submission, through one of two NIH protocols, depending on their donor source. Consent to these protocols is optional and does not affect the recipient's access to transplant or to unrelated donor grafts. For unrelated donor graft recipients, this data submission element ensures compliance with standards described in the Transplant Center Agreement between the NIH Clinical Center and the NMDP.

**End of study procedures:** Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

**Loss or destruction of data:** Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

## 5.2 RESPONSE CRITERIA

Standard disease specific clinical and laboratory response criteria will be employed as detailed in **Appendix 9** and **Appendix 10**.

## 5.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

## 5.4 DATA REPORTING EXCEPTIONS

For patients who meet off-treatment criteria, but remain on-study and who are undergoing treatment of toxicity **related to the investigational therapy** (e.g., second transplant for treatment of graft rejection), only adverse events will be recorded and reported for those events which are > grade 3 or serious, are previously unexpected AND are deemed possibly, probably or definitely related to a procedure, test, or treatment, or require intervention. Adverse events resulting from other disease-related treatments not associated with this investigational therapy (i.e. treatment for progression of disease), or from events not related to the patient's disease will NOT be recorded or reported.

Expedited reporting will not apply to events arising from participation in another clinical trial or alternative treatment for disease progression.

# 6 STATISTICAL CONSIDERATIONS

## 6.1 SUBJECT ACCRUAL

Subjects of both genders, from all racial and ethnic groups are eligible for this trial if they meet the criteria outlined in Section **2.1**. To date, there is no information that suggests differences in disease response among racial or ethnic groups or between the genders, indicating that results of the trial will be applicable to all groups. Efforts will be made to extend the accrual to a representative population, but in a pilot study with limited accrual, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, age, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

## 6.2 STATISTICS AND FEASIBILITY

The first primary objective of this study is to assess the feasibility and toxicity associated with infusion of escalating doses of donor derived natural killer (NK) cells (NK-DLI) on Days 21 (± 3 days) and 49 days (± 7 days) following infusion 1, following allogeneic HLA-matched T cell depleted (TCD) peripheral blood stem cell transplantation (PBSCT) in patients with metastatic or recurrent pediatric solid tumors and leukemias. Because of the experience in the first 8 patients transplanted, recipients will now be divided into 2 cohorts: 1) matched related donor recipients and 2) unrelated donor recipients. Patients will be enrolled onto this trial using a conventional Phase I dose escalation design in which 3 patients per dose level, in each cohort are entered, with 3 additional patients enrolled at the dose level, following the usual guidelines for a phase I trial, based on the development of DLTs. After enrollment of the first six patients on study without DLT, cohort 1 was expanded by a maximum of 6 patients to study the pathophysiology of symptoms observed to date. Because no GVHD was seen in related donor recipients (n = 4), dose level 1 for related donor recipients will be closed and patients with related donors will start accrual onto dose level 2. Since the expansion of dose level 1, one additional unrelated donor recipient had been transplanted and developed grade 4 GVHD. Although the GVHD was steroid responsive and therefore did not meet definition of DLT, we find the incidence and severity of the GVHD in these patients unacceptable. Therefore, all subsequent recipients will be treated with GVHD prohylaxis and patients with unrelated donors will continue to enroll on cell dose level 1. In addition to assessment for DLT, if >1 of the additional 3 patients with unrelated donors, enrolled at the cohort 1 cell dose level experience Grade 3 or any experience grade 4 acute GVHD, the NK cell dose level will not be escalated without further consultation with the FDA and IRB regarding a protocol modification.

Consistent with a phase I design, the highest dose level at which 0/6 or 1/6 patients has a DLT will be expanded. If at any dose level, there are 2 or more patients who experience a DLT, then no further dose escalations will occur. At the expanded dose level, if 3 or more of 9, or 4 or more of 12 patients experience a DLT, consideration for de-escalation and an expanded cohort at a lower dose level will be given through a protocol amendment. The feasibility objective would be met if >=80% of patients receive 2 doses of NK cell infusions within 56 days of HSCT. As described in the protocol, patients are scheduled to receive NK-DLI on Days 21 (± 3 days) and 49 (± 7 days) but the doses may be delayed based upon clinical judgment of the treating team or

concern for toxicity related to previous infusions. Moreover, it is possible that patients may not receive adequate cells because of toxicity, product failure, or product contamination.

A second primary objective of this trial is to determine if full donor lymphoid engraftment in T cell depleted, MHC matched sibling or unrelated donor transplants with NK-DLI can be accomplished using this regimen. Donor engraftment is defined as >95% donor lymphoid chimerism (CD3+ T-cells in peripheral blood) on post-BMT day +100. The highest dose explored will enroll 12 total patients in order to allow adequate numbers of patients to receive the regimen for more accurate estimates of engraftment, aGVHD and toxicity at that dose level. Because NK cells are presumed to enhance engraftment rates, only patients enrolled on the highest dose level will be evaluable for the engraftment endpoint. If however, at any dose level, engraftment is problematic and primary graft failures occur in 2 patients, the protocol stopping rule for insufficient engraftment described below will be triggered. It would be desirable to have greater than 75% of patients at the highest NK dose level show sustained donor lymphoid engraftment (>95% at day 100). Thus, if 8 or more of 12 patients engraft, this will be considered adequate since the upper one sided 90% CI about 7/12 is 0.781.

A third primary objective of this trial is to determine rates of aGVHD in TCD, MHC matched sibling or unrelated donor transplants given NK-DLI. Upon completion of the trial, the incidence of all aGVHD will be reported with appropriate confidence intervals at all dose levels. All histologies will be analyzed together for this end point, since the incidence of aGVHD is not likely to be influenced by this variable. In addition, however, we will evaluate the incidence of aGVHD as it relates to histology for exploratory purposes in an attempt to identify any unexpected relationships that might exist. A rate of <25% grade III or grade IV acute GVHD per cohort would be required to consider this approach of interest for further development. All patients enrolled, regardless of NK dose level, will be evaluable for the aGVHD endpoint. If however, excessive aGVHD is observed, this will trigger the stopping rules described below.

## 6.3 EARLY STOPPING RULES

If the number of patients who have ever been treated on this trial at any dose level 1) develop steroid refractory grade 3 or grade 4 aGVHD at any time point, 2) show primary graft failure - defined as failure to achieve independence from platelet transfusion or ANC never reaching > 500 cells/mcl by day 35 post-BMT, or 3) experience treatment related mortality within 100 days (defined as toxicity from the preparative regimen, infection, aGVHD or therapy for aGVHD), exceeds 1/5 (upper 90% confidence interval of 58%), 3/10 (upper 90% CI of 55%), or 4/15 (upper 90% CI of 46%) or 6/20 (upper 90% CI of 47%), then accrual to the trial will terminate as soon as this can be evaluated, regardless of how successful the clinical outcomes have been, as this will be considered excessive toxicity.

In addition, if a single patient (**exception**: patients with bcr-abl+ leukemia) develops a positive bcr-abl PCR confirmed by repeat testing, there will be a temporary suspension to accrual and no products infusions will be given to patients currently on study.

## 6.4 SUBJECT ACCRUAL GOAL

As stated in section **3.1**, three to 6 recipients of unrelated donors will be treated on cell dose level 1, and three to 6 recipients will be treated on cell dose 2 per cohort, with an additional six recipients enrolled on cohort 3, or the highest cohort explored. Thus, a total of up to 38 recipients (and 38 donors) may be enrolled on this trial. Prior to enrolling on the first cohort, a single

patient will receive DLI-NK cells once ANC reaches at least 1000 cells/µL or within 42 days following transplant. Due to toxicities observed in the first patient, a second patient received this regimen. Once these first two patients had undergone transplant and accomplished hematopoietic recovery, cohort 1 was opened 28 days after the last NK cell infusion. With a rare possibility of not achieving the targeted cell numbers per cohort, an additional 3 recipient/donor pairs may be enrolled if needed to replace patients who do not meet the targeted cell dose for the assigned cohort, for a maximum of 43 recipients and 43 donors. With an approximately 5-7 patients enrolling per year, anticipated accrual is 3-4 years with 3 year post-transplant follow up. It is therefore likely this trial will remain open for approximately 6 - 8 years.

# 7 HUMAN SUBJECTS PROTECTIONS

## 7.1 RATIONALE FOR SUBJECT SELECTION

### 7.1.1 Recipients

Allogeneic hematopoietic stem cell transplants are being performed at increasing rates each year for patients with malignant diseases. Patients with hematologic malignancies will be the subjects for this study. This patient group was chosen because current treatment options offer little to no hope for survival and new approaches to therapy are desperately needed. While the diseases targeted in this trial affect all races, and genders and subjects of both genders, efforts will be made to extend accrual to a representative population, but in a small pilot trial, it may be difficult if not impossible to achieve complete balance in this regard. The trial will be listed on the NCI and NIH websites and clinical trials search sites. Individuals with HIV disease will not be candidates for this protocol, due to the high risk of post-transplant complications in this population. Individuals who are pregnant or lactating will not be candidates for this protocol, due to risk to the fetus or newborn.

### 7.1.2 Donors

Related donors will be first-degree HLA-matched family members of the transplant recipient. In most cases the best donor is a sibling. All donors will be selected in accordance with NIH CC Department of Transfusion Medicine standards. Minors will only be selected when they represent the most suitable stem cell donor for the individual recipient. In such cases, the oldest suitable donor will be selected and approval to participate will require medical review by a pediatric practitioner, evaluation by a social worker, and psychological assessment by a mental health specialist with pediatric expertise. For donors who are minors, informed consent from their legal guardian and donor verbal assent will be obtained.

Unrelated donors will be HLA-matched (at least 9/10 allele) and will be selected in accordance with National Marrow Donor Program (NMDP) standards.

## 7.2 PARTICIPATION OF CHILDREN

The age range of patients eligible for this trial is 4 to 35 years of age. Physicians, nurses, and multidisciplinary support teams have expertise in the management of children undergoing PBSCT. All children enrolled on this protocol will be followed and cared for in the Pediatric Oncology Branch clinic.

## 7.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

### 7.3.1 Recipients

Survival for the population of patients eligible for this trial is limited. This population has a very high risk of dying from their disease, and other therapeutic options are primarily experimental. Although the overall treatment plan is experimental, the PBSCT portion of this study is an acceptable therapeutic option for patients in this population, and pre-clinical immunologic studies indicate there may be some benefit to the addition of NK cell administration. The goal of this study is to determine the safety and feasibility of this approach and to determine if transplantation of allogeneic (related or unrelated) HLA-matched NK cells post PBSCT can result in full donor engraftment (>95% by day 100), while reducing the incidence of Graft-versus-Host Disease (GVHD), and reducing the risks associated with infection. Patients participating in this trial will be treated with therapeutic intent, and the response to therapy will be monitored. The potential benefits from PBSCT alone are prolonged disease remission and/or reduction in cancer-related symptoms, while the potential additional benefits of the NK cell infusion are unknown at this time. This protocol involves greater than minimal risk, but does present the potential for direct benefit to individual subjects according to 21CFR50.52, 46.405.

7.3.1.1 Risks associated with PBSCT

The primary risks to patients participating in this research study include toxicity of the chemotherapy and immunosuppressive drugs and allogeneic transplantation. The chemotherapy utilized in this study has the risk of direct drug toxicity. In addition, the immune suppression resulting from this therapy is associated with an increased risk of infections. The primary risks associated with allogeneic stem cell transplantation are graft rejection, GVHD and regimen related toxicity. This protocol utilizes a profoundly immunosuppressive induction and preparative regimen designed to facilitate rapid hematologic engraftment. Indeed, patients treated with a similar regimen and T cell depleted graft in the ETIB have shown >90% myeloid engraftment in the majority of patients by day 28 and in all patients by day 98. In addition, the POB and the ETIB have extensive experience with this induction and preparative regimen with T cell replete grafts and have seen universally brisk engraftment, therefore graft rejection is not anticipated to be limiting. In this regimen, melphalan has been added for additional cytoreductive effects. While this may increase the probability that the preparative regimen is myeloablative, it lessens the chance for graft rejection even further and thus we believe that graft rejection is very unlikely in this protocol.

Thus, the evidence suggests that GVHD and tumor recurrence/progression will represent the primary cause of morbidity and mortality post-transplant. Because of the history of significant rates of GVHD in previous POB trials, the graft will be T cell depleted and the risk for GVHD is likely to be far lower. The protocol provides for detailed and careful monitoring of all patients to assess for toxicity and response to treatment. All patients entered on the trial will have high risk pediatric malignancy for which standard therapy is ineffective. Further, the chemotherapy drugs used on this protocol are known to be effective for the types of cancer that these patients will have.

7.3.1.2 Risks associated with allogeneic NK cell administration

In previous studies, grade 2 and 3 anemias have been reported though most patients had anemia at study entry. Neutropenia has also been reported, but this was following chemotherapy agents. There were no episodes of neutropenic fever and thrombocytopenia was not observed. Hematologic toxicity was transient and there was no occurrence of prolonged cytopenia or marrow aplasia. Reports suggest that engraftment is improved, rates of GVHD and infections are

lower with NK cell infusions, however, a negative impact on engraftment and the development of GVHD are theoretic risks.

Based on experiences on this trial, however, there is an apparent increase in GVHD in recipients of unrelated donor products in comparison to recipients of related donor products, which is thought to be at least possibly related to the allogeneic NK cell administration. Because of this disparity, several changes have been made to the protocol to ameliorate the potential risk of NK related GVHD. 1) NK cells are now given no earlier than day 21 (+/- 3) days post PBSCT to avoid concurrent engraftment syndrome which may be exacerbated by NK cell administration; 2) Recipients of unrelated donor products will receive immunosuppression to ameliorate the potential risk of GVHD.

7.3.1.3 Tumor biopsy

In the event a patient has a readily accessible tumor, adult patients may be asked to undergo biopsy in order to obtain tissue for research evaluation (see section **3.2.2**). Standard techniques will be used for percutaneous biopsies and may include CT and / or ultrasound guidance. Although direct benefit from research conducted on this tumor biopsy is unlikely, participation in this research may allow patients some benefit in knowing their contribution may lead to a greater understanding of their cancer and potential benefit to others in the future. Patients enrolled on this protocol suffer from extremely rare malignancies with very low survival rates and often have a strong desire to participate in research that may lead to a better understanding of their disease.

7.3.2 Donors

The donor stem cell collection procedure on this trial is conducted per standard clinical routine.

There are a number of potential benefits to donors who participate on this trial. The most probable is psychological benefit from contributing to medical care designed to improve the health of another. Donors appreciate that their participation represents an essential component to a transplant, which is performed with curative intent. This psychological benefit may be derived both at the time of donation, as well as in the future, since it is hoped that the transplant will result in prolongation and/or improvement in the quality of the recipient's life. Donor participation may also help advance scientific knowledge about stem cell transplantation and lead to improvements in the treatment of cancer. (213)

The main risks to donors are the possible side effects of stem cell harvesting. Stem cell donation is a safe procedure that is routinely performed in healthy children and adults. The potential side effects of filgrastim used for mobilization are outlined in Section **9.8**. The most common side effects of apheresis are pain and bruising at IV sites. Side effects of a temporary central venous catheter, required for young children, include pain, bleeding, bruising, infection, thrombosis, and vascular perforation. Mild side effects from citrate anticoagulant are common and include chills, numbness and tingling sensations ("pins and needles"), anxiety, muscle cramps, and nausea. More serious side effects due to citrate-induced low calcium levels are uncommon and include low blood pressure, seizures, weakness, and tetany. Citrate reactions rapidly resolve away when the collection is slowed down or stopped. Transient mild thrombocytopenia is common after donation. To prevent dilutional anemia, the extracorporeal circuit must be primed with 1 unit of red blood cells for small children. Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. There is the possibility that the donor will have negative psychological effects (e.g., guilt, sadness) if the transplant is unsuccessful or if complications

(e.g. GVHD) contribute to recipient morbidity or mortality. Donors will be closely monitored and procedures to minimize risks and prevent side effects are incorporated into all aspects of the protocol. The POB, DTM, and NIH CC and NMDP Donor Sites have broad expertise to adequately manage side effects, including in pediatric donors (related donors only).

## 7.4 RISK/BENEFIT ANALYSIS

All patients enrolled on this study are at very high risk of disease recurrence and from dying of their disease. Patients entered on the trial will be treated with therapeutic intent and response to the therapy and possible toxicity will be closely monitored. Patients on this study may directly benefit from the chemotherapy administered as it may provide anti-tumor activity. Successful allogeneic PBSCT may offer patients a curative therapy for their cancer. It is hypothesized that the addition of NK cell infusions may improve engraftment, reduce the incidence of GVHD and lower infection risks; although these results are not known at this time.

Therefore, this protocol involves greater than minimal risk to patients, but presents the potential for direct benefit to individual subjects and therefore meets Federal requirements for patients that are minors. (45CFR46.405)

## 7.5 CONSENT PROCESSES AND DOCUMENTATION

### 7.5.1 Recipients

A signed informed consent document will be obtained prior to entry onto the study. The PI or an associate investigator on the trial will obtain consent. The PI, an Associate Investigator, or their designee will be available to answer all questions from patients and their parents or guardians. The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and potential benefits, and alternative therapies will be carefully explained to the patient and/or the patient's parents or guardian if he/she is of minor age.

The investigators are requesting a waiver from the IRB to allow only one parent to sign the informed consent to enter a child on the protocol. Because many patients must travel to the NIH from long distances at substantial expense, requiring both parents to be present for the consent process could be a financial hardship for many families. When guardianship status of the child is uncertain, documentation of custody status must be obtained.

Where deemed appropriate by the clinician and the child's parent(s) or guardian, the child will also be included in all discussions about the trial and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. Written assent will not be obtained from children as the study holds out the prospect of direct benefit that is important to the health and well-being of the child and is available only in the context of the research. Verbal assent will be obtained as appropriate for children ages > 7 and the parent or guardian will sign the designated line on the informed consent attesting to the fact that the child has given assent. Children under the age of 7 will not be required to provide assent as they typically do not have the cognitive ability to fully understand the nature of research. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide assent (verbal versus written) as applicable. All children will be contacted after they have reached the age of 18 to

determine whether they wish to continue on the trial and informed consent will be obtained from them at that time.

7.5.2 Related donors

Informed consent will also be obtained from all donors entered on this trial. Donors < 18 years of age will be evaluated by a social worker and mental health specialist (psychologist or psychiatrist) prior to the assent process to determine willingness to participate. If willingness to participate has been confirmed, verbal-assent, as described above, will also be obtained. The attached informed consent documents contain all elements required for consent. In addition, the Principal Investigator, Associate Investigator, or their designee will be available to answer all questions from patients, donors, and their parents or guardians.

7.5.3 Unrelated Donors

Informed consent will be obtained from all unrelated donors entered on this trial. This process will be conducted at the NMDP Donor Center using the Protocol Informed Consent Document for Unrelated Donors, which will be approved by both the NCI and NMDP IRBs. Per NMDP regulations, and to maintain donor confidentiality, the signed informed consent document will *not* be sent to the NIH. Instead, the NMDP will issue a certification notice to the NIH confirming donor consent.

7.5.4 Consent for minors when they reach the age of majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Given the length of time that has transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who have completed their participation in the research study.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.
(3) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only plan to request a waiver of reconsent for those subjects who have been lost to follow-up or who, prior to the approval of Amendment Q, have been taken off study prior to reaching the age of majority.

### 7.5.5 Telephone consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

# 8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

## 8.1 DEFINITIONS

### 8.1.1 Adverse Events

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

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### 8.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

### 8.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

### 8.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

### 8.1.5 Serious adverse events

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

8.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

8.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

8.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

8.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

## 8.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

8.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

8.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

   a) All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;

   b) All Grade 3 and 4 events that are possibly, probably or definitely related to the research;

   c) All Grade 5 events regardless of attribution;

   d) All Serious Events regardless of attribution;

   NOTE: Grade 1 events are not required to be reported.

8.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

### 8.3 IND SPONSOR REPORTING CRITERIA

During the first 30 days after the subject receives investigational agent/intervention, the investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only report those that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events including deaths due to progressive disease must be reported within one business day

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

8.3.1 Action Plan for Positive Results on Cell Product Safety Testing

8.3.1.1 In the unlikely event that a positive sterility test or mycoplasma test result is obtained after distribution of the cell product, or after administration of the product to the patient, the following steps will be initiated IMMEDIATELY:

a. CPS, DTM personnel will notify the principal investigator, Dr. Nirali Shah, MD at (240) 760-6199 or by pager 301-496-1211. As soon as the identification and sensitivity report from positive sterility is available, a copy of the final report will be sent to the PI. The DTM QS officer and CPS Supervisor will determine the need for quality improvement based on the nature and extent of the incident.
b. If CPS is unable to reach Dr. Shah within 15 minutes, contact the page operator (301-496-1211) to page the Pediatric Oncology Branch Fellow on-call. NOTE: Dr. Shah or the POB Fellow on-call will contact the attending physician for the POB Service, who will determine the extent of the work-up of a positive culture in consultation with staff from the Microbiology Service.
c. Dr. Shah /attending physician will discuss the positive results with the patient/parents, and specify the clinical therapy, antibiotic regimen and/or monitoring plan in consultation with staff from the Microbiology Service.
d. A contaminated sample of a product that has been administered to a patient will be handled in the same fashion as a Grade 4 toxicity (CTC version 4.0) and the Sponsor will be responsible for notifying the NCI IRB and the FDA. A full written report with description of events, laboratory findings, clinical evaluation and treatments would be submitted to the NCI IRB within 7 working days. The Sponsor will be responsible for filing this report with the FDA within 15 calendar days.

## 8.4 DATA SAFETY MONITORING PLAN

8.4.1 Principal Investigator/Research Team

The Principal Investigator will review all adverse events. The Principal Investigator will meet with the research team at frequent and regularly scheduled intervals, to determine treatment modifications and treatment based toxicities, initiating enrollment to the next lower weight based group, to monitor trends in adverse events, and determine if trends are noted.

Adverse events will be tracked by the PI. If trends are noted and/or risks warrant it, accrual will be interrupted and/or the protocol and/or consent document will be amended accordingly. If trends in toxicities are noted or stopping rules are met, the PI will temporarily suspend enrollment while reviewing the episodes with the NCI IRB and FDA to determine if any changes to the protocol or the informed consent are warranted. At the completion of each cohort, a toxicity summary with both acute and delayed toxicities will be formally reported to the FDA prior to opening the subsequent cohort. In addition, if a toxicity occurs after D 28 in any patient, that meets DLT criteria, is serious or is unexpected, it will be reported to the FDA immediately, and when applicable, discussions with the FDA and IRB regarding dose adjustments will occur.

The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8.4.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects' protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

#### 8.4.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

## 9 PHARMACEUTICAL INFORMATION

### 9.1 FLUDARABINE (FLUDARA)

Availability: Fludarabine monophosphate is commercially available and will be supplied by the Clinical Center Pharmacy. FLUDARA IV, is supplied as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludara is stored at room temperature.

Storage and Stability: Reconstituted FLUDARA IV is chemically and physically stable for 24 hours at room temperature, or for 48 hours if refrigerated. Because reconstituted FLUDARA IV contains no antimicrobial preservative, care must be taken to assure the sterility of the prepared solution; for this reason, reconstituted FLUDARA IV should be used or discarded within 8 hours.

Preparation: FLUDARA IV should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, 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 7-8.5. The product may be further diluted for intravenous administration in 50 ml of 5% Dextrose for Injection USP, or in 0.9% Sodium Chloride, USP.

Administration: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine will be mixed in 50 ml of 0.9% NaCl, and infused i.v. over 30 minutes.

Toxicity: Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgias, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia, autoimmune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status has occurred after very high doses (approximately 4 times higher than the standard doses employed in this protocol). Severe neurologic toxicity has only rarely been demonstrated at the 25-30 mg/m$^2$/day dosage of fludarabine. Rarely describe complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agents.

### 9.2 MESNA (SODIUM 2-MERCAPTOETHANESULFONATE, MESNEX)

Availability: Commercially available and will supplied by the Clinical Center Pharmacy.

Stability: Mesna may be diluted in D5W, 0.9%NaCl, or D5/0.45NaCl to concentrations between 0.6 mg/ml and 20 mg/ml for intravenous infusions. These concentrations are stable at room temperature for 24 hours.

Administration: Will be administered by continuous i.v. infusion on days –6, -5, -4, and –3 of the transplant during high dose cyclophosphamide administration. Dose of mesna will be 1200 mg/m$^2$ per day by continuous i.v. infusion for four consecutive days (days –6, -5, -4, and –3).

Toxicity May be associated with nausea, vomiting, diarrhea, or headaches.

### 9.3 CYCLOPHOSPHAMIDE (CYTOXAN)

Availability: Commercially available as a lyophilized powder in 2 gram vials and will supplied by the Clinical Center Pharmacy.

Storage and Stability: Following reconstitution as directed, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8C.

Preparation: Reconstitution with 100 ml of sterile water for injection to a final concentration of 20 mg/ml.

Administration: Cyclophosphamide will be diluted in 0.9% NaCl and infused over 30 minutes during induction and 2-hours during pre-HSCT preparative therapy.

Toxicity: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea, and vomiting occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Mesna has been used prophylactically as an uroprotective agent in patients receiving cyclophosphamide. Prophylactic

mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration.

### 9.4 ETOPOSIDE (VEPESID, VP-16)

Availability: Commercially available and will be supplied by the Clinical Center Pharmacy.

Etoposide Toxicity: Includes nausea, vomiting, stomatitis, diarrhea, neutropenia, thrombocytopenia, and alopecia. Secondary AML has been associated with etoposide. Bradycardia and hypotension are sometimes observed with etoposide administration.

### 9.5 DOXORUBICIN (ADRIAMYCIN)

Availability: Commercially available and will be supplied by the Clinical Center Pharmacy.

Doxorubicin Toxicity: Includes nausea, vomiting, stomatitis, myelosuppression, and cardiotoxicity. Cardiotoxicity is particularly noted after cumulative doses of greater than 550 $mg/m^2$. Other toxicities include nausea, vomiting, stomatitis, diarrhea, and alopecia. Skin infiltration of doxorubicin causes tissue necrosis.

### 9.6 VINCRISTINE

Availability: Commercially available and will be supplied by the Clinical Center Pharmacy.

Vincristine Toxicity Vincristine causes neurological toxicities such as paresthesias, jaw pain, ataxia, foot-drop, cranial nerve palsies, paralytic ileus, constipation, abdominal pain, and loss of deep tendon reflexes. It is also a vesicant, and occasionally causes alopecia and myelosuppression. Because fluconazole can delay the clearance of vincristine, fluconazole will be discontinued during days 1, 2, 3, and 4 of induction chemotherapy.

Storage and Stability: Stability studies conducted by the Pharmaceutical Development Service, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride for Injection (at concentrations, respectively, of 1, 25, and 125 mcg/ml; 2, 50, and 250 mcg/ml; and 2.8, 70, and 350 mcg/ml) are stable for at least 36 hours at room temperature when protected from light.

Administration of these Agents as Continuous Infusion: In this study, the daily dose of vincristine, doxorubicin, and etoposide (i.e. the 24 hour supply) will be admixed together in 500 ml of 0,9% Sodium Chloride Injection and delivered with a suitable infusion pump through a

central venous access device. All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine PLUS an overfill (excess) fluid and a proportional amount of drug to compensate for volume lost in administration set tubing to ensure delivery of the entire 24 hour dose. Before dispensing 3-in-1 admixtures, Pharmacy staff will 1) purge all air from the drug product container, 2) attach an administration set appropriate for use with a portable pump and the set will be 3) primed close to its distal tip and 4) capped with a Luer-locking cap. Bags will be exchanged daily for 4 consecutive days to complete a 96-hour drug infusion. Portable pumps used to administer etoposide + doxorubicin + vincristine admixtures will be programmed to deliver 528 ml per day at 22 ml/hr. At the end of an infusion, some residual fluid is expected because overfill fluid and drug were added; however, nurses are asked to return to the Pharmacy for measurement any drug containers that appear to contain a greater amount of residual drug than expected.

### 9.7 PREDNISONE

Availability: Commercially available and will be supplied by the Clinical Center Pharmacy; available in solid or liquid dosage forms. Tablet strengths include 1, 2.5, 5, 20, and 50 mg forms, and the oral solution is at 1 mg/ml.

Administration: Prednisone will be administered at a dose of 60 mg/m$^2$ orally in 2-4 divided doses on days 1, 2, 3, 4, and 5 of fludarabine-EPOCH induction chemotherapy regimen. In patients unable to tolerate oral medication, methylprednisolone can be substituted at the same dosage, diluted in 25 ml of D5W, and infused over 15 minutes. To reduce gastrointestinal side effects, prednisone should be taken with food.

Toxicity: Prednisone frequently causes gastritis or ulcers, hypertension, immunosuppression, muscle wasting, fluid retention, increased appetite, psychiatric disturbances, hyperglycemia, and skin changes. In addition, steroids can also cause pancreatitis, impaired growth, menstrual abnormalities, osteoporosis, osteonecrosis, cataracts, pseudotumor cerebri, glaucoma, and seizures.

### 9.8 FILGRASTIM (G-CSF, NEUPOGEN)

Availability: Filgrastim is a commercially available and will be supplied by the Clinical Center Pharmacy. This product differs from the natural form due to its absence of N-terminal o-glycosylation. Filgrastim has undergone tests for USP pyrogen, limulus amebocyte assay, sterility and the general safety test. The nucleic acid content can be no greater than 10 pg/unit dose. The final product is clear, colorless sterile solution that is free of particulates; the product is not less than 95% pure. It is provided in 1 ml vials of 300 mcg or 1.6 ml vials of 480 mcg.

Storage and Stability: Intact vials should be stored in the refrigerator; do not freeze. Product is stable for at least 1 year when refrigerated.

Preparation: Do not dilute with sodium chloride solutions.

Administration: Inject subcutaneously at 5-10 mcg/kg/day as per treatment plan.

Toxicity: Filgrastim may cause bone pain, constitutional symptoms such as fever, fatigue and myalgias, splenomegaly, thinning hair, pain at the injection site, bone pain, worsening of pre-existing inflammatory conditions, and elevations in uric acid, LDH, and alkaline phosphatase.

### 9.9 PEGFILGRASTIM (RECOMBINANT METHIONYL HUMAN G-CSF, NEULASTA™)

Availability: Pegfilgrastim is a commercially available and will be supplied by the Clinical Center Pharmacy. Pegfilgrastim is a covalent conjugate of recombinant methionyl human G-CSF (filgrastim) and monomethoxypolyethylene glycol. To produce pegfilgrastim, a 20 kD monomethoxypolyethylene glycol molecule is covalently bound to the N-terminal methionyl residue of filgrastim. The average molecular weight of pegfilgrastim is approximately 39 kD. Pegfilgrastim is supplied in 0.6 mL prefilled syringes for subcutaneous injection, containing 6 mg pegfilgrastim (based on protein weight), in a sterile, clear, colorless, preservative-free solution (pH 4.0) containing acetate (0.35 mg), sorbitol (30.0 mg), polysorbate 20 (0.02 mg), and sodium (0.02 mg) in water for injection, USP.

Storage and Stability: Pegfilgrastim should be stored refrigerated and protected from light until time of use. Shaking or freezing should be avoided. Before injection, Pegfilgrastim may be allowed to reach room temperature for a maximum of 48 hours but should be protected from light.

Administration: Inject subcutaneously as per treatment plan.

Toxicity: See filgrastim above.

### 9.10 CYTARABINE (CYTOSINE ARABINOSIDE, CYTOSAR, ARA-C)

Availability: Commercially available.

Storage and Stability: The reconstituted drug is stable for 48 hours at room temperature. The drug will be further diluted in an age appropriate amount of 5% Dextrose Injection or 0.9% Sodium Chloride Injection prior to administration.

Administration: Cytarabine is available for intravenous and intrathecal administration. The manufacturer supplies the drug as unreconstituted lyophilized powder in vials. With each vial, an ampule of bacteriostatic water for injection containing 0.945% benzyl alcohol is provided. To prepare high dose (2 gm/$m^2$) cytarabine intravenous infusions, each vial of drug should be reconstituted with 2 cc (100 mg) or 10 cc (500 mg) of sterile water for injection USP (i.e. not the manufacturer-provided diluent) which contains no preservative. Begin each cytarabine dose 3.5 hours after completion of the preceding fludarabine dose.

Toxicity: IV Administration: Acute DLT consists of severe leukopenia and thrombocytopenia. Nausea and vomiting may be dose limiting at higher doses. Other adverse reactions include diarrhea, immunosuppression, anorexia, stomatitis, oral ulceration, flu-like syndrome, fever, hepatic dysfunction, and alopecia. At high doses, as in this protocol, keratoconjunctivitis, dermatitis, and central nervous system toxicity (e.g., ataxia, somnolence, coma, dysarthria) may occur. Occasionally, the CNS impairment is not fully reversible. Renal impairment will enhance toxicity. In the event signs of CNS toxicity, the cytarabine will be interrupted and the M.D. notified. In the event of signs of CNS toxicity, the cytarabine infusion will be interrupted and the M.D. notified. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine.

Conjunctivitis Prophylaxis: Corticosteroid ophthalmic drops will be administered 2 drops to each eye every 6 hours starting prior to first dose and continuing until 24 hours after the last dose of cytarabine has been completed.

## 9.11 INTRATHECAL CHEMOTHERAPY

9.11.1 Methotrexate (MTX, amethopterin) NSC #000740

Route: Intrathecal therapy (methotrexate single agent)

Source and Pharmacology: A folate analogue that inhibits the enzyme dihydrofolate reductase, halting DNA, RNA, and protein synthesis. Initial IV half-life is about 1.2 hours, with a second phase of 10.4 hours. About 50% is bound to protein. Transport into the cell is carrier-mediated. Once in the cell, MTX (Glu)n are formed, the number of which are related to the cytocidal effect. Once MTX (Glu)n are formed, they do not pass back out of the cell unless converted back to MTX. After oral administration, about 60% of a 30 mg/m2 dose is rapidly absorbed from the GI tract, with peak blood levels at 1 hour. Above this dose, absorption decreases significantly. Absorption can be very erratic, varying between 23% to 95%. A 20-fold difference between peak levels of drug has been reported (0.1 to 2mM). There is significant enterohepatic circulation of MTX: 9% of MTX is excreted in feces. MTX is excreted unchanged in the urine, except at high doses when it is partially metabolized to hydroxy-MTX and excreted.

Formulation and Stability: Intact vials may be stored at room temperature (22°-25°C) and are stable for at least 2 years or until date of expiration. INTRATHECAL MTX: Available in various dosages in preservative-free liquid, 25mg/ml in a 2ml vial, or as a lyophilized powder. Reconstitute the powder with buffered saline solution. The Methotrexate solutions may be further diluted with buffered saline or the patient's own CSF. After mixing it should be used within 24 hours, since MTX contains no antibacterial preservative.

Guidelines for Administration: The drug will be filtered and mixed in sterile preservative free saline less than 4 hours prior to administration. Intrathecal delivery should be approximately isovolumetric (ml CSF out = ml drug in) via lumbar puncture (LP) in the lateral decubitus position. The patient should remain in prone or Trendelburg position for approximately 30 minutes post LP to facilitate drug circulation throughout the CNS. Leucovorin rescue should be administered 5 mg PO/IV q 6 hours x 4 doses, starting 24 hours after methotrexate.

Supplier: Commercially available. See package insert for further information.

Toxicities: The following toxicities may occur when methotrexate is given alone into the spinal fluid:

| | **Common**<br>Happens to 21-100 children out of every 100 | **Occasional**<br>Happens to 5-20 children out of every 100 | **Rare**<br>Happens to < 5 children out of every 100 |
|---|---|---|---|
| **Immediate:**<br>Within 1-2 days of receiving drug | | Headache, abnormally high number of cells in the spinal fluid | Vomiting, fever, rash, drowsiness, stiff neck, irritation of tissues in the brain/spinal cord, seizures (L), partial paralysis |
| **Prompt:** | | | Decrease in the number of red and white blood cells and platelets made in the bone marrow, |

| | | | |
|---|---|---|---|
| Within 2-3 weeks, prior to the next course | | | drowsiness, unsteady walk |
| **Delayed:**<br>Any time later during therapy | | Learning disability | Thinning of nerve fiber insulation resulting in difficulty with loss of memory, concentration, balance and walking, which may get worse with time[1] (L) |
| **Late:**<br>Any time after the completion of treatment | | | Increasingly poor nervous system function[1] |

[1] ***May be enhanced by HDMTX and/or cranial irradiation.***

***(L) Toxicity may also occur later.***

Methotrexate crosses the placenta to the fetus. Fetal toxicities and teratogenic effects of methotrexate (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: congenital defects, chromosome abnormalities, malformation, severe newborn myelosuppression, pancytopenia, and low birth weight.

Dose Modification**:** Hold for any CNS toxicity.

9.11.2 Hydrocortisone (Cortef, Solu-cortef)

Route: Intrathecal therapy (as clinically indicated)

Source and Pharmacology: Commercially available. See package insert for further information. Synthetic steroid akin to natural adrenal hormone, cortisol. It binds with steroid receptors on nuclear membrane, impairs cellular mitosis and inhibits protein synthesis. It is phase specific, killing cells primarily during S phase. It has a catabolic effect on proteins and alters the kinetics of peripheral blood leukocytes. It is excreted in the urine and catabolized in the liver.

Toxicity: The following toxicities may occur when hydrocortisone is given intrathecally: nausea and vomiting, headache, pleocytosis, fever, somnolence, meningismus, learning disability, leukoencephalopathy.

Formulation and Stability: Available as 100 mg, 250 mg, 500 mg, and 1000 mg vials for aqueous injection. In powder form, the drug is stable for 2 years at room temperature. After reconstitution, it is stored at room temperature, and should be discarded after 3 days.

Intrathecal Administration: IT hydrocortisone should be reconstituted with physiologic buffered diluents (lactated Ringer's, 0.9% sodium chloride, Elliott's B solution). Do not use Bacteriostatic Water to reconstitute for IT use; use only preservative free solutions.

Guidelines for Administration: Hydrocortisone will be given by IT administration in an age-specified dose and will be mixed with cytarabine.

9.11.3 Cytarabine (cytosine arabinoside, AraC, Cytosar)

Route: Intrathecal therapy (as clinically indicated)

Source and Pharmacology: Commercially available. See package insert for further information. Deoxycytidine analogue which is metabolized to ARA-CTP, a substance which inhibits DNA polymerase. It is S phase specific, and thus affects DNA synthesis.

Toxicity: The following toxicities may occur when cytarabine is given intrathecally: Nausea, vomiting, headache, pleocytosis, arachnoiditis, rash, fever, somnolence, meningismus, convulsions, paresis, myelosuppression, ataxia, learning disability. CNS impairment may not be fully reversible. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine.

Formulation and Stability: A freeze-dried powder available in 100 mg, 500 mg, 1 G and 2 G vials. The unreconstituted form of the drug is stable at room termperature for at least 2 years.

Intrathecal Administration: IT cytarabine should be reconstituted with physiologic buffered diluents (lactated Ringer's, 0.9% sodium chloride, Elliott's B solution). Do not use Bacteriostatic Water to reconstitute for IT use; use only preservative free solutions.

Guidelines for Administration: Cytarabine will be given by IT administration in an age-specified dose and will be mixed with hydrocortisone.

### 9.12 RANITIDINE (ZANTAC):

Will be given as a pre-medication. It will be supplied by the Clinical Center Pharmacy. The most common side effects are fatigue, dizziness, headache, and diarrhea. See package insert for additional information.

### 9.13 ACETAMINOPHEN (TYLENOL):

Will be given as a pre-medication. It will be supplied by the Clinical Center Pharmacy. The most common side effect seen with high doses or chronic usage is hepatotoxicity. Rarely, sensitivity reactions can occur. See package insert for additional information.

### 9.14 DIPHENHYDRAMINE (BENADRYL):

Will be given as a pre-medication IV over 10-15 minutes. It will be supplied by the Clinical Center Pharmacy. The most common side effects are sleepiness, dizziness, restlessness, and irritability. See package insert for additional information.

### 9.15 FLUCONAZOLE (DIFLUCAN):

Will be given as prophylaxis against susceptible yeast and fungi. It will be supplied by the Clinical Center Pharmacy. Fluconazole increases the AUC and serum concentration of tacrolimus and sirolimus. Thus, careful monitoring of levels is important when these medications are administered concurrently. Significant interactions with oral hypoglycemics, coumarin-type anticoagulants, phenytoin, rifampin, theophylline, cisapride, astemizole, rifabutin and tacrolimus have been observed. As described in section **3.3.2.7**, interactions with vincristine also occur. Rare mild side effects including headache, nausea and abdominal pain have been reported. Elevations in transaminases can occur. Hepatotoxicity is usually, but not always, reversible. Fluconazole has been associated with rare cases of severe hepatic toxicity, including fatalities. Other adverse effects include anaphylaxis, and rash (occasionally severe). See package insert for additional information.

## 9.16 PCP PROPHYLAXIS

9.16.1 Trimethoprim/Sulfamethoxazole (TMP/SMX) (Bactrim, Septra):

Will be given as prophylaxis against pneumocystis. It will be provided by the Clinical Center Pharmacy. Side effects include a reduction in blood counts, gastrointestinal disturbances (nausea, vomiting and anorexia) and allergic reactions. Interactions with thiazide diuretics, phenytoin, and methotrexate occur and require caution when used concurrently. Due to photosensitivity, patients should avoid prolonged exposure to the sun. Fatalities associated with the administration of sulfonamides, although rare, have occurred due to Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis and blood dyscrasias. See package insert for additional information.

9.16.2 Pentamadine:

Will be given as prophylaxis against pneumocystis, as a substitute for TMP/SMX. It will be provided by the Clinical Center Pharmacy. It is supplied in 300 mg per nebulizer. Dosing will be as per the CC BMT Guidelines (http://intranet.cc.nih.gov/bmt/clinicalcare/pdf/Table_VII.pdf). Side effects reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia. IV formulation may be used if there is a contraindication to inhaled delivery.

## 9.17 INTRAVENOUS IMMUNOGLOBULIN (IVIG, GAMIMMUNE, GAMMAGARD, SANDOGLOBULIN):

Will be used for infection prophylaxis in the post-transplant period. It will be provided by the Clinical Center Pharmacy and will be administered by standard guidelines. Side effects vary depending on the preparation but can included anaphylactic reactions, hypotension, fever, chills, headache, urticaria, and aseptic meningitis. In general, these effects are infusion-related and can be treated by decreasing the rate of infusion. Rarely, acute renal failure has been reported, predominantly in individuals with pre-existing renal dysfunction. Although donor screening and inactivation procedures substantially reduce the risk of viral transmission, immune globulin is made from human plasma and, therefore, carries a risk of transmission of infectious agents. See package insert for the individual preparations for additional information.

## 9.18 PALIFERMIN, KEPIVANCE™ (KGF)

Kepivance™ (palifermin) is a human keratinocyte growth factor (KGF) produced by recombinant DNA technology in *Escherichia coli (E coli)*. It will be supplied by the Clinical Center Pharmacy. See the package insert for complete details.

Palifermin is supplied as a sterile, white, preservative-free, lyophilized power for IV injection after reconstitution with 1.2 mL of sterile water for injection, USP. Palifermin will be administered to decrease the incidence and duration of oral mucositis. It may be administered at 60 mcg/kg/day IV bolus injection for 3 days, twice:

1. The first 3 daily doses should be administered prior to the preparative chemotherapy, with the 3rd dose at least 24 hours prior to starting the preparative regimen,
2. The last 3 daily doses should be administered starting on day -1.

Toxicities associated with use of palifermin include: rash, erythema, edema, pruritus, dysesthesia, tongue discoloration, tongue thickening, alteration of taste, pain arthralgias and dysesthesia.

### 9.19 RECOMBINANT HUMAN INTERLEUKIN 15 (IL-15)

Source and Pharmacology:

rhIL-15 will be purchased by the POB Branch from CellGenix™ for use in cell preparation. rhIL-15 is produced under GMP conditions for investigational use; the source is a DNA sequence encoding the mature IL-15 protein expressed in *E. coli.* **For clinical *ex vivo* cell culture use only.**

Formulation and Stability:

Lyophilized from a 250 mcg/mL, 0.2 mcm-sterile filtered solution in PBS (300 mM NaCl) + 0.1% Tween 20. Each vial contains 50 mcg (+/- 10%). Product is stable for 2 years from date of production when stored as directed. This study will require approximately 2 vials per patient for cell development.

Storage: Store vials at -20°C to -80°C; avoid repeated freeze-thaw cycles.

### 9.20 CYCLOSPORINE A

Availability: Cyclosporine A is commercially available as either an injectable concentrate or as a microemulsion in capsules or liquid. Capsules are available in 25 and 100 mg strengths. Liquid solution is available as 100 mg/ml.

Storage and Stability: Stable at room temperature.

Administration: Cyclosporine will be started by IV infusion at day –1 before the transplant at a dose of 2 mg/kg/dose (over a two hour period) for two doses per day. Once the patient is able to tolerate oral feedings, CSA will be converted to an equivalent oral dose. The starting oral dose should be approximately 2-times the IV dose given in a q 12 hour schedule. The dose should be titrated to a trough level of 150-300 ng/ml (monoclonal RIA methodology).

Drug Interactions Cyclosporine has important interactions with multiple medications. Most commonly, CSA levels are altered due to induction or inhibition of hepatic metabolism. Azole antifungal agents (e.g., fluconazole), macrolides (e.g., erthyromycin), ciprofloxacin, metronidazole, grapefruit juice, cimetidine, metoclopramide, oral contraceptive pills, and corticosteroids may result in elevated cyclosporin A levels. Agents that decrease CSA levels include nafcillin, anticonvulsants (e.g., phenytoins, barbituates), and octreotide. CSA levels should be monitored more closely when any such agents are co-administered, in the setting of impaired renal or hepatic function, and when other nephrotoxic or hepatoxic drugs are used. Cisapride is contraindicated due to risk of QT prolongation and arrhythmias.

Toxicity: Includes nephrotoxic effects, which are manifested as increases in BUN and creatinine, hypertension, hyperkalemia, and hyperuricemia; nephrotoxicity is increased by co-administration of acyclovir, aminoglycoside antibiotics, and amphotericin B. Nervous system effects include a fine hand tremor, seizures, headache, paresthesis, flushing, and confusion. Cyclosporine-induced seizures may be associated with concurrent high-dose corticosteroid therapy, hypertension, and hypomagnesemia. Rarely, cyclosporine can induce a syndrome of severe neurotoxicity characterized by cortical blindness, quadriplegia, seizures, and coma. Adverse dermatologic

effects include hirsutism and gingival hyperplasia. Elevated liver transaminases may occur. Adverse GI effects include diarrhea, nausea and vomiting, anorexia and abdominal discomfort.

### 9.21 TACROLIMUS (FK506, PROGRAF)

Supply: Tacrolimus will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (0.5 mg, 1mg, and 5mg), and as a parenteral concentrate for injection (5 mg/ml, 1 ml ampules).

Preparation: For parenteral doses, tacrolimus injection concentrate (5 mg/ml) should be diluted to a final concentration of 0.004 to 0.02 mg/ml in dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of tacrolimus will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing.

Storage and Stability: Capsules and ampules of parenteral concentrate bear expiration dates and are stored at room temperature. Tacrolimus concentrate for injection that has been diluted to a final concentration of 0.004 to 0.02 mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.

Administration: Tacrolimus may be given intravenously over 24 hours or orally.

Toxicities: Acute tacrolimus nephrotoxicity is usually manifested by a moderate decline in renal excretory function, which is readily reversible by a decrease in drug dosage. Although some degree of transient renal dysfunction may occur in patients with therapeutic levels of tacrolimus, significant renal toxicity is associated with elevated trough or steady state levels. In addition to an increase in BUN and creatinine, hyperkalemic hyperchloremic acidosis, low fractional excretion of sodium and the onset of hypertension with hypomagnesemia are seen with tacrolimus nephrotoxicity. Insulin dependent post transplant diabetes mellitus was reported in 20% kidney transplant patients, which was reversible in 50% of the patients at 2 years post transplant. Hypertension occurs in up to 60% of patients. Hypomagnesaemia can be associated with neurological symptoms, including seizures, cerebellar ataxia and depression. Dose-related hepatotoxicity, manifested by elevation of serum transaminases and bilirubin, has been reported. The drug carries a warning for increased susceptibility to infection and the possible development of lymphoma may result from resulting immunosuppression.

### 9.22 BUSULFAN (BUSULFEX)

General: Busulfex is a bifunctional alkylating agent approved for use as a conditioning agent prior to allogeneic hematopoieitc stem cell transplantation. The IV formulation is administered as Busulfex (Busulfex, Otsuka America Phamraceutical, Inc.)

Supply: For patient administration, IV Busulfex is purchased by the NIH Clinical Center Pharmacy Department from commercial sources. The drug is supplied as a clear, colorless sterile solution in 10ml single use vials. Each vial of BUSULFEX contains 60 mg (6mg/ml) of busulfan.

Storage and Stability: Unopened vials of BUSUILFEX are stable until the date indicated on the package when stored under refrigeration at 2-6 degrees C (36-46 degrees F).

Administration: BUSULFEX must be diluted prior to use with either 0.9% Sodium chloride or 5% Dextrose Injection. The diluent quantity should be 10 times the volume of BUSULFEX so that the final concentration of busulfan is approximately 0.5 mg/ml. BUSULFEX should be administered intravenously via a central venous catheter as a two hour infusion every 24 hours for a total of 4 doses over four days.

Toxicities: 1) At the indicated dose and schedule BUSULFEX induces profound myelosuppression; 2) All patients should be pre-medicated with phenytoin or Clonazepam plus Levetiracetam (Keppra) since busulfan is known to cross the blood brain barrier and induce seizures; 3) Nausea, vomiting, and stomatitis are common side-effects of busulfan.

Drug Interactions: Itraconazole increases the clearance of busulfan by up to 25%. Phenytoin increases the clearance of busulfan by 15%.

### 9.23 LEVETIRACETAM (KEPPRA)

Supply: 500 mg tablets

Preparation: None

Storage and Stability: Tablets are stored at room temperature.

Route of Administration: Orally. Children: the dose is 10 mg/kg/dose PO BID (maximum dose: 1000 mg). Adults: drug will be given as 500mg po every 12 hours. Dosing starts the night before BUSULFEX and continues until 24 hours after last Busulfan dose.

Toxicities: Leukopenia, hepatic abnormalities.

### 9.24 LOREZAPAM (ATIVAN)

General: benzodiazepine

Administration: Oral or IV, 0.02-0.05 mg/kg/dose, maximum dose: 2 mg) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose.

Toxicity: CNS depression, confusion, paroxysimal agitation.

### 9.25 RADIATION THERAPY

9.25.1 TOTAL BODY IRRADIATION

Chemical name: Not applicable

Known toxicities: The side effects of radiation have been well described. The most common include nausea and mucositis. There also exists a risk of hypothyroidism, cataracts, interstitial pneumonitis, nephropathy, and an unspecified long-term risk of developing secondary malignancies. Importantly, the majority of the non-neoplastic effects were subclinical and/or reversible. Studies attempting to evaluate the risk attributed to radiation within a preparative regimen have implicated secondary malignancies, however this is difficult to ascribe since secondary malignancies are also increased with cGVHD and other post-transplant complications. Pediatric patients who require sedation in order to undergo TBI will be asked to sign a separate consent outlining the risk of the selected anesthesia.

9.25.2 CNS Boost

Chemical name: Not applicable

Known toxicities: For the CNS boost, the common short-term side effects include skin redness and irritation in the area treated, hair loss in the area treated, fatigue, and uncommonly headaches, weakness, numbness, or other neurologic deficit which may require steroid treatment, nausea, vomiting, weight loss, and rarely decrease blood cell count and excessive need of sleep. Long-term side effects of whole-brain radiation therapy, which may occur months to years after, are mild decrease in memory or ability to think (which may be exacerbated in those who have received prior radiation therapy to the brain), scalp discoloration and thickening, permanent thinning of the hair in the treated area. Uncommonly, severe memory loss, cataracts, permanent loss of brain cells, seizures, hearing loss. Rarely, death of brain cells can cause swelling in the brain and require long-term steroid treatment, hospitalization and/or surgery, loss or decrease in vision. Extremely rare is development of new tumors in the scalp, skull or brain.

## 10 REFERENCES

1. Bosi A, Laszlo D, Labopin M, Reffeirs J, Michallet M, Gluckman E, et al. Second allogeneic bone marrow transplantation in acute leukemia: results of a survey by the European Cooperative Group for Blood and Marrow Transplantation. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(16):3675-84.

2. Bishop MR, Steinberg SM, Gress RE, Hardy NM, Marchigiani D, Kasten-Sportes C, et al. Targeted pretransplant host lymphocyte depletion prior to T-cell depleted reduced-intensity allogeneic stem cell transplantation. British journal of haematology. 2004;126(6):837-43.

3. Hardy NM, Hakim F, Steinberg SM, Krumlauf M, Cvitkovic R, Babb R, et al. Host T cells affect donor T cell engraftment and graft-versus-host disease after reduced-intensity hematopoietic stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2007;13(9):1022-30.

4. Conrad R, Remberger M, Cederlund K, Ringden O, Barkholt L. A comparison between low intensity and reduced intensity conditioning in allogeneic hematopoietic stem cell transplantation for solid tumors. Haematologica. 2008;93(2):265-72.

5. Carvallo C, Childs R. Nonmyeloablative stem cell transplantation as immunotherapy for kidney cancer and other metastatic solid tumors. Cytotechnology. 2003;41(2-3):197-206.

6. Demirer T, Barkholt L, Blaise D, Pedrazzoli P, Aglietta M, Carella AM, et al. Transplantation of allogeneic hematopoietic stem cells: an emerging treatment modality for solid tumors. Nat Clin Pract Oncol. 2008;5(5):256-67.

7. Verneris MR, Wagner JE. Recent developments in cell-based immune therapy for neuroblastoma. Journal of neuroimmune pharmacology. 2007;2(2):134-9.

8. Ladenstein R, Lasset C, Hartmann O, Klingebiel T, Bouffet E, Gadner H, et al. Comparison of auto versus allografting as consolidation of primary treatments in advanced neuroblastoma over one year of age at diagnosis: report from the European Group for Bone Marrow Transplantation. Bone marrow transplantation. 1994;14(1):37-46.

9. Kanold J, Paillard C, Tchirkov A, Merlin E, Marabelle A, Lutz P, et al. Allogeneic or haploidentical HSCT for refractory or relapsed solid tumors in children: toward a neuroblastoma model. Bone Marrow Transplant. 2008;42 Suppl 2:S25-30.

10. Inoue M, Nakano T, Yoneda A, Nishikawa M, Nakayama M, Yumura-Yagi K, et al. Graft-versus-tumor effect in a patient with advanced neuroblastoma who received HLA haplo-identical bone marrow transplantation. Bone marrow transplantation. 2003;32(1):103-6.

11. Hirayama M, Azuma E, Araki M, Komada Y, Nakagawa A. Evidence of graft-versus-tumor effect in refractory metastatic neuroblastoma. Transplantation. 2006;82(1):142-4.

12. Lucas K, Schwartz C, Kaplan J. Allogeneic stem cell transplantation in a patient with relapsed Ewing sarcoma. Pediatric blood & cancer. 2008;51(1):142-4.

13. Capitini CM, Derdak J, Hughes MS, Love CP, Baird K, Mackall CL, et al. Unusual sites of extraskeletal metastases of Ewing sarcoma after allogeneic hematopoietic stem cell transplantation. J Pediatr Hematol Oncol. 2009;31(2):142-4.

14. Bregni M, Bernardi M, Ciceri F, Peccatori J. Allogeneic stem cell transplantation for the treatment of advanced solid tumors. Springer Seminars in Immunopathology. 2004;26(1-2):95-108.

15. Burdach S, van Kaick B, Laws HJ, Ahrens S, Haase R, Korholz D, et al. Allogeneic and autologous stem-cell transplantation in advanced Ewing tumors: An update after long-term follow-up from two centers of the European Intergroup Study EICESS. Ann Oncol. 2000;11(11):1451-62.

16. Koscielniak E, Gross-Wieltsch U, Treuner J, Winkler P, Klingebiel T, Lang P, et al. Graft-Versus-Ewing Sarcoma Effect and Long-Term Remission Induced by Haploidentical Stem-Cell Transplantation in a Patient With Relapse of Metastatic Disease. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(1):242-4.

17. Doelken R, Weigel S, Schueler F, Doelken G, Beck JF. Poor outcome of two children with relapsed state stage IV alveolar rhabdomyosarcoma after allogeneic stem cell transplantation. Pediatric hematology and oncology. 2005;22(8):699-703.

18. Misawa A, Hosoi H, Tsuchiya K, Iehara T, Sawada T, Sugimoto T. Regression of refractory rhabdomyosarcoma after allogeneic stem-cell transplantation. Pediatric hematology and oncology. 2003;20(2):151-5.

19. Donker AE, Hoogerbrugge PM, Mavinkurve-Groothuis AMC, van de Kar NCAJ, Boetes C, Hulsbergen-van de Kaa CA, et al. Metastatic rhabdomyosarcoma cured after chemotherapy and allogeneic SCT. Bone marrow transplantation. 2009;43(2):179-80.

20. Matthay KK, Seeger RC, Reynolds CP, Stram DO, O'Leary MC, Harris RE, et al. Allogeneic versus autologous purged bone marrow transplantation for neuroblastoma: a report from the Childrens Cancer Group. Journal of clinical oncology. 1994;12(11):2382-9.

21. Shen BJ, Hou HS, Zhang HQ, Sui XW. Unrelated, HLA-mismatched multiple human umbilical cord blood transfusion in four cases with advanced solid tumors: initial studies. Blood cells. 1994;20(2-3):285-92.

22. Lang P, Pfeiffer M, Müller I, Schumm M, Ebinger M, Koscielniak E, et al. Haploidentical stem cell transplantation in patients with pediatric solid tumors: preliminary results of a pilot study and analysis of graft versus tumor effects. Klinische Pädiatrie. 2006;218(6):321-6.

23. Dean RM, Fowler DH, Wilson WH, Odom J, Steinberg SM, Chow C, et al. Efficacy of reduced-intensity allogeneic stem cell transplantation in chemotherapy-refractory non-hodgkin lymphoma. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2005;11(8):593-9.

24. Bishop MR, Hou JW, Wilson WH, Steinberg SM, Odom J, Castro K, et al. Establishment of early donor engraftment after reduced-intensity allogeneic hematopoietic stem cell transplantation to potentiate the graft-versus-lymphoma effect against refractory lymphomas. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2003;9(3):162-9.

25. Fowler D, Hou J, Foley J, Hakim F, Odom J, Castro K, et al. Phase I clinical trial of donor T-helper type-2 cells after immunoablative, reduced intensity allogeneic PBSC transplant. Cytotherapy. 2002;4(5):429-30.

26. Chamberlain MC, Prados MD, Silver P, Levin VA. A phase II trial of oral melphalan in recurrent primary brain tumors. Am J Clin Oncol. 1988;11(1):52-4.

27. Appelbaum FR, Buckner CD. Overview of the clinical relevance of autologous bone marrow transplantation. Clin Haematol. 1986;15(1):1-18.

28. Gonzalez-Llano O, Jaime-Perez J, Cantu-Rodriguez O, Mancias-Guerra C, Gutierrez-Aguirre H, Herrera-Garza J, et al. Successful father-to-son stem cell transplantation in a child with hemophagocytic lymphohistiocytosis using a reduced-intensity conditioning regimen. Eur J Haematol. 2006;77(4):341-4.

29. Llano OG, Perez JC, Rodriguez OC, Guerra CM, Aguirre HG, Garza JL, et al. Allogeneic hematopoietic stem cell transplantation using a reduced-intensity conditioning regimen in infants: experience at a single institution in Mexico. Pediatr Hematol Oncol. 2008;25(1):39-47.

30. Hahn T, Wall D, Camitta B, Davies S, Dillon H, Gaynon P, et al. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of acute lymphoblastic leukemia in children: an evidence-based review. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2005;11(11):823-61.

31. Miser JS, Goldsby RE, Chen Z, Krailo MD, Tarbell NJ, Link MP, et al. Treatment of metastatic Ewing sarcoma/primitive neuroectodermal tumor of bone: evaluation of increasing the dose intensity of chemotherapy--a report from the Children's Oncology Group. Pediatric blood & cancer. 2007;49(7):894-900.

32. Paulussen M, Ahrens S, Burdach S, Craft A, Dockhorn-Dworniczak B, Dunst J, et al. Primary metastatic (stage IV) Ewing tumor: survival analysis of 171 patients from the EICESS studies. European Intergroup Cooperative Ewing Sarcoma Studies. Ann Oncol. 1998;9(3):275-81.

33. Pinkerton CR, Bataillard A, Guillo S, Oberlin O, Fervers B, Philip T. Treatment strategies for metastatic Ewing's sarcoma. Eur J Cancer. 2001;37(11):1338-44.

34. Institute NC. PDQ Summary. http://wwwcancergov/cancertopics. 2009.

35. Hayes-Jordan A, Doherty DK, West SD, Raney RB, Blakely ML, Cox CS, Jr., et al. Outcome after surgical resection of recurrent rhabdomyosarcoma. J Pediatr Surg. 2006;41(4):633-8; discussion -8.

36. Lal DR, Su WT, Wolden SL, Loh KC, Modak S, La Quaglia MP. Results of multimodal treatment for desmoplastic small round cell tumors. J Pediatr Surg. 2005;40(1):251-5.

37. Gray JC, Kohler JA. Immunotherapy for neuroblastoma: turning promise into reality. Pediatric blood & cancer. 2009;53(6):931-40.

38. Cotterill SJ, Pearson AD, Pritchard J, Kohler JA, Foot AB. Late relapse and prognosis for neuroblastoma patients surviving 5 years or more: a report from the European Neuroblastoma Study Group "Survey". Medical and pediatric oncology. 2001;36(1):235-8.

39. Mertens AC, Yasui Y, Neglia JP, Potter JD, Nesbit ME, Jr., Ruccione K, et al. Late mortality experience in five-year survivors of childhood and adolescent cancer: the Childhood Cancer Survivor Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(13):3163-72.

40. Navid F, Armstrong M, Barfield RC. Immune therapies for neuroblastoma. Cancer Biol Ther. 2009;8(10):874-82.

41. Castel V, Canete A, Melero C, Acha T, Navajas A, Garcia-Miguel P, et al. Results of the cooperative protocol (N-III-95) for metastatic relapses and refractory neuroblastoma. Medical and pediatric oncology. 2000;35(6):724-6.

42. Creutzig U, Ritter J, Zimmermann M, Reinhardt D, Hermann J, Berthold F, et al. Improved treatment results in high-risk pediatric acute myeloid leukemia patients after intensification with high-dose cytarabine and mitoxantrone: results of Study Acute Myeloid Leukemia-Berlin-Frankfurt-Munster 93. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(10):2705-13.

43. Stevens RF, Hann IM, Wheatley K, Gray RG. Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukemia: results of the United Kingdom Medical Research Council's 10th AML trial. MRC Childhood Leukaemia Working Party. British journal of haematology. 1998;101(1):130-40.

44. Perel Y, Auvrignon A, Leblanc T, Vannier JP, Michel G, Nelken B, et al. Impact of addition of maintenance therapy to intensive induction and consolidation chemotherapy for childhood acute myeloblastic leukemia: results of a prospective randomized trial, LAME 89/91. Leucamie Aique Myeloide Enfant. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(12):2774-82.

45. Capizzi RL, Poole M, Cooper MR, Richards F, 2nd, Stuart JJ, Jackson DV, Jr., et al. Treatment of poor risk acute leukemia with sequential high-dose ARA-C and asparaginase. Blood. 1984;63(3):694-700.

46. Woods WG, Neudorf S, Gold S, Sanders J, Buckley JD, Barnard DR, et al. A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission. Blood. 2001;97(1):56-62.

47. Feig SA, Lampkin B, Nesbit ME, Woods WG, Versteeg CM, Buckley JD, et al. Outcome of BMT during first complete remission of AML: a comparison of two sequential studies by the Children's Cancer Group. Bone Marrow Transplant. 1993;12(1):65-71.

48. Burnett AK, Goldstone AH, Stevens RM, Hann IM, Rees JK, Gray RG, et al. Randomised comparison of addition of autologous bone-marrow transplantation to intensive chemotherapy for acute myeloid leukaemia in first remission: results of MRC AML 10 trial. UK Medical Research Council Adult and Children's Leukaemia Working Parties. Lancet (London, England). 1998;351(9104):700-8.

49. Razzouk BI, Estey E, Pounds S, Lensing S, Pierce S, Brandt M, et al. Impact of age on outcome of pediatric acute myeloid leukemia: a report from 2 institutions. Cancer. 2006;106(11):2495-502.

50. Ortega JJ, Madero L, Martin G, Verdeguer A, Garcia P, Parody R, et al. Treatment with all-trans retinoic acid and anthracycline monochemotherapy for children with acute promyelocytic leukemia: a multicenter study by the PETHEMA Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(30):7632-40.

51. Testi AM, Biondi A, Lo Coco F, Moleti ML, Giona F, Vignetti M, et al. GIMEMA-AIEOPAIDA protocol for the treatment of newly diagnosed acute promyelocytic leukemia (APL) in children. Blood. 2005;106(2):447-53.

52. De Botton S, Chevret S, Sanz M, Dombret H, Thomas X, Guerci A, et al. Additional chromosomal abnormalities in patients with acute promyelocytic leukaemia (APL) do not confer poor prognosis: results of APL 93 trial. British journal of haematology. 2000;111(3):801-6.

53. Fenaux P, Chevret S, Guerci A, Fegueux N, Dombret H, Thomas X, et al. Long-term follow-up confirms the benefit of all-trans retinoic acid in acute promyelocytic leukemia. European APL group. Leukemia. 2000;14(8):1371-7.

54. Ravindranath Y, Abella E, Krischer JP, Wiley J, Inoue S, Harris M, et al. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. Blood. 1992;80(9):2210-4.

55. Gamis AS, Woods WG, Alonzo TA, Buxton A, Lange B, Barnard DR, et al. Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(18):3415-22.

56. Massey GV. Transient leukemia in newborns with Down syndrome. Pediatric blood & cancer. 2005;44(1):29-32.

57. Oliansky DM, Rizzo JD, Aplan PD, Arceci RJ, Leone L, Ravindranath Y, et al. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of acute myeloid leukemia in children: an evidence-based review. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2007;13(1):1-25.

58. Amadori S, Testi AM, Arico M, Comelli A, Giuliano M, Madon E, et al. Prospective comparative study of bone marrow transplantation and postremission chemotherapy for childhood acute myelogenous leukemia. The Associazione Italiana Ematologia ed Oncologia Pediatrica Cooperative Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1993;11(6):1046-54.

59. Dahl GV, Kalwinsky DK, Mirro J, Jr., Look AT, Pui CH, Murphy SB, et al. Allogeneic bone marrow transplantation in a program of intensive sequential chemotherapy for children and young adults with acute nonlymphocytic leukemia in first remission. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1990;8(2):295-303.

60. Nesbit ME, Jr., Buckley JD, Feig SA, Anderson JR, Lampkin B, Bernstein ID, et al. Chemotherapy for induction of remission of childhood acute myeloid leukemia followed by marrow transplantation or multiagent chemotherapy: a report from the Childrens Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1994;12(1):127-35.

61. Wells RJ, Woods WG, Buckley JD, Odom LF, Benjamin D, Bernstein I, et al. Treatment of newly diagnosed children and adolescents with acute myeloid leukemia: a Childrens Cancer Group study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1994;12(11):2367-77.

62. Aladjidi N, Auvrignon A, Leblanc T, Perel Y, Benard A, Bordigoni P, et al. Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(23):4377-85.

63. Watson M, Buck G, Wheatley K, Homewood JR, Goldstone AH, Rees JK, et al. Adverse impact of bone marrow transplantation on quality of life in acute myeloid leukaemia patients; analysis of the UK Medical Research Council AML 10 Trial. Eur J Cancer. 2004;40(7):971-8.

64. Cassileth PA, Harrington DP, Appelbaum FR, Lazarus HM, Rowe JM, Paietta E, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. The New England journal of medicine. 1998;339(23):1649-56.

65. Zittoun RA, Mandelli F, Willemze R, de Witte T, Labar B, Resegotti L, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Leukemia Cooperative Groups. The New England journal of medicine. 1995;332(4):217-23.

66. Burnett AK, Wheatley K, Goldstone AH, Stevens RF, Hann IM, Rees JH, et al. The value of allogeneic bone marrow transplant in patients with acute myeloid leukaemia at differing risk of relapse: results of the UK MRC AML 10 trial. British journal of haematology. 2002;118(2):385-400.

67. Chen AR, Alonzo TA, Woods WG, Arceci RJ. Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation?--an American view. British journal of haematology. 2002;118(2):378-84.

68. Creutzig U, Reinhardt D. Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation?--a European view. British journal of haematology. 2002;118(2):365-77.

69. Smith FO, Alonzo TA, Gerbing RB, Woods WG, Arceci RJ. Long-term results of children with acute myeloid leukemia: a report of three consecutive Phase III trials by the Children's Cancer Group: CCG 251, CCG 213 and CCG 2891. Leukemia. 2005;19(12):2054-62.

70. Ravindranath Y, Chang M, Steuber CP, Becton D, Dahl G, Civin C, et al. Pediatric Oncology Group (POG) studies of acute myeloid leukemia (AML): a review of four consecutive childhood AML trials conducted between 1981 and 2000. Leukemia. 2005;19(12):2101-16.

71. Smith M, Arthur D, Camitta B, Carroll AJ, Crist W, Gaynon P, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1996;14(1):18-24.

72. McLean TW, Ringold S, Neuberg D, Stegmaier K, Tantravahi R, Ritz J, et al. TEL/AML-1 dimerizes and is associated with a favorable outcome in childhood acute lymphoblastic leukemia. Blood. 1996;88(11):4252-8.

73. Borkhardt A, Cazzaniga G, Viehmann S, Valsecchi MG, Ludwig WD, Burci L, et al. Incidence and clinical relevance of TEL/AML1 fusion genes in children with acute

lymphoblastic leukemia enrolled in the German and Italian multicenter therapy trials. Associazione Italiana Ematologia Oncologia Pediatrica and the Berlin-Frankfurt-Munster Study Group. Blood. 1997;90(2):571-7.

74. Uckun FM, Pallisgaard N, Hokland P, Navara C, Narla R, Gaynon PS, et al. Expression of TEL-AML1 fusion transcripts and response to induction therapy in standard risk acute lymphoblastic leukemia. Leukemia & lymphoma. 2001;42(1-2):41-56.

75. Kanerva J, Saarinen-Pihkala UM, Niini T, Riikonen P, Mottonen M, Makipernaa A, et al. Favorable outcome in 20-year follow-up of children with very-low-risk ALL and minimal standard therapy, with special reference to TEL-AML1 fusion. Pediatric blood & cancer. 2004;42(1):30-5.

76. Johansson B, Moorman AV, Haas OA, Watmore AE, Cheung KL, Swanton S, et al. Hematologic malignancies with t(4;11)(q21;q23)--a cytogenetic, morphologic, immunophenotypic and clinical study of 183 cases. European 11q23 Workshop participants. Leukemia. 1998;12(5):779-87.

77. Pui CH, Frankel LS, Carroll AJ, Raimondi SC, Shuster JJ, Head DR, et al. Clinical characteristics and treatment outcome of childhood acute lymphoblastic leukemia with the t(4;11)(q21;q23): a collaborative study of 40 cases. Blood. 1991;77(3):440-7.

78. Crist W, Pullen J, Boyett J, Falletta J, van Eys J, Borowitz M, et al. Clinical and biologic features predict a poor prognosis in acute lymphoid leukemias in infants: a Pediatric Oncology Group Study. Blood. 1986;67(1):135-40.

79. Reaman G, Zeltzer P, Bleyer WA, Amendola B, Level C, Sather H, et al. Acute lymphoblastic leukemia in infants less than one year of age: a cumulative experience of the Children's Cancer Study Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1985;3(11):1513-21.

80. Arico M, Valsecchi MG, Camitta B, Schrappe M, Chessells J, Baruchel A, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. The New England journal of medicine. 2000;342(14):998-1006.

81. Crist WM, Carroll AJ, Shuster JJ, Behm FG, Whitehead M, Vietti TJ, et al. Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): a Pediatric Oncology Group study. Blood. 1990;76(1):117-22.

82. Uckun FM, Sensel MG, Sather HN, Gaynon PS, Arthur DC, Lange BJ, et al. Clinical significance of translocation t(1;19) in childhood acute lymphoblastic leukemia in the context of contemporary therapies: a report from the Children's Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1998;16(2):527-35.

83. Moorman AV, Richards SM, Martineau M, Cheung KL, Robinson HM, Jalali GR, et al. Outcome heterogeneity in childhood high-hyperdiploid acute lymphoblastic leukemia. Blood. 2003;102(8):2756-62.

84. Charrin C, Thomas X, Ffrench M, Le QH, Andrieux J, Mozziconacci MJ, et al. A report from the LALA-94 and LALA-SA groups on hypodiploidy with 30 to 39 chromosomes and near-triploidy: 2 possible expressions of a sole entity conferring poor prognosis in adult acute lymphoblastic leukemia (ALL). Blood. 2004;104(8):2444-51.

85. Heerema NA, Sather HN, Sensel MG, Zhang T, Hutchinson RJ, Nachman JB, et al. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (> 50 chromosomes). Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2000;18(9):1876-87.

86. Sutcliffe MJ, Shuster JJ, Sather HN, Camitta BM, Pullen J, Schultz KR, et al. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. Leukemia. 2005;19(5):734-40.

87. Harrison CJ, Moorman AV, Broadfield ZJ, Cheung KL, Harris RL, Reza Jalali G, et al. Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. British journal of haematology. 2004;125(5):552-9.

88. Heerema NA, Nachman JB, Sather HN, Sensel MG, Lee MK, Hutchinson R, et al. Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report from the children's cancer group. Blood. 1999;94(12):4036-45.

89. Raimondi SC, Zhou Y, Mathew S, Shurtleff SA, Sandlund JT, Rivera GK, et al. Reassessment of the prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. Cancer. 2003;98(12):2715-22.

90. Pui CH, Carroll AJ, Raimondi SC, Land VJ, Crist WM, Shuster JJ, et al. Clinical presentation, karyotypic characterization, and treatment outcome of childhood acute lymphoblastic leukemia with a near-haploid or hypodiploid less than 45 line. Blood. 1990;75(5):1170-7.

91. Chessells JM. Relapsed lymphoblastic leukaemia in children: a continuing challenge. British journal of haematology. 1998;102(2):423-38.

92. Gaynon PS, Harris RE, Altman AJ, Bostrom BC, Breneman JC, Hawks R, et al. Bone marrow transplantation versus prolonged intensive chemotherapy for children with acute lymphoblastic leukemia and an initial bone marrow relapse within 12 months of the completion of primary therapy: Children's Oncology Group study CCG-1941. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(19):3150-6.

93. Raetz EA, Borowitz MJ, Devidas M, Linda SB, Hunger SP, Winick NJ, et al. Reinduction platform for children with first marrow relapse of acute lymphoblastic Leukemia: A Children's Oncology Group Study[corrected]. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(24):3971-8.

94. Nguyen K, Devidas M, Cheng SC, La M, Raetz EA, Carroll WL, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. Leukemia. 2008;22(12):2142-50.

95. Ritchey AK, Pollock BH, Lauer SJ, Andejeski Y, Barredo J, Buchanan GR. Improved survival of children with isolated CNS relapse of acute lymphoblastic leukemia: a pediatric oncology group study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1999;17(12):3745-52.

96. Buchanan GR, Boyett JM, Pollock BH, Smith SD, Yanofsky RA, Ghim T, et al. Improved treatment results in boys with overt testicular relapse during or shortly after initial

therapy for acute lymphoblastic leukemia. A Pediatric Oncology group study. Cancer. 1991;68(1):48-55.

97. Barrett AJ, Horowitz MM, Pollock BH, Zhang MJ, Bortin MM, Buchanan GR, et al. Bone marrow transplants from HLA-identical siblings as compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission. The New England journal of medicine. 1994;331(19):1253-8.

98. Balduzzi A, Valsecchi MG, Uderzo C, De Lorenzo P, Klingebiel T, Peters C, et al. Chemotherapy versus allogeneic transplantation for very-high-risk childhood acute lymphoblastic leukaemia in first complete remission: comparison by genetic randomisation in an international prospective study. Lancet (London, England). 2005;366(9486):635-42.

99. Sadowitz PD, Smith SD, Shuster J, Wharam MD, Buchanan GR, Rivera GK. Treatment of late bone marrow relapse in children with acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood. 1993;81(3):602-9.

100. Ravandi F, Kebriaei P. Philadelphia chromosome-positive acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2009;23(5):1043-63, vi.

101. Pulsipher MA. Treatment of CML in pediatric patients: should imatinib mesylate (STI-571, Gleevec) or allogeneic hematopoietic cell transplant be front-line therapy? Pediatric blood & cancer. 2004;43(5):523-33.

102. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. The New England journal of medicine. 2006;355(23):2408-17.

103. Shah NP. Loss of response to imatinib: mechanisms and management. Hematology Am Soc Hematol Educ Program. 2005:183-7.

104. Kolb EA, Pan Q, Ladanyi M, Steinherz PG. Imatinib mesylate in Philadelphia chromosome-positive leukemia of childhood. Cancer. 2003;98(12):2643-50.

105. Saussele S, Lauseker M, Gratwohl A, Beelen DW, Bunjes D, Schwerdtfeger R, et al. Allogeneic hematopoietic stem cell transplantation (alloSCT) for chronic myeloid leukemia in the imatinib era; evaluation of its impact within a subgroup of the randomized German CML Study IV. Blood. 2009.

106. Goldman JM, Marin D. Management decisions in chronic myeloid leukemia. Semin Hematol. 2003;40(1):97-103.

107. Wassmann B, Pfeifer H, Scheuring U, Klein SA, Gokbuget N, Binckebanck A, et al. Therapy with imatinib mesylate (Glivec) preceding allogeneic stem cell transplantation (SCT) in relapsed or refractory Philadelphia-positive acute lymphoblastic leukemia (Ph+ALL). Leukemia. 2002;16(12):2358-65.

108. Weisdorf DJ, Anasetti C, Antin JH, Kernan NA, Kollman C, Snyder D, et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. Blood. 2002;99(6):1971-7.

109. Gratwohl A, Hermans J, Goldman JM, Arcese W, Carreras E, Devergie A, et al. Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow

transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Lancet (London, England). 1998;352(9134):1087-92.

110. Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C, et al. An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. Blood. 1999;94(5):1517-36.

111. Stary J, Locatelli F, Niemeyer CM. Stem cell transplantation for aplastic anemia and myelodysplastic syndrome. Bone Marrow Transplant. 2005;35 Suppl 1:S13-6.

112. Niemeyer CM, Kratz CP, Hasle H. Pediatric myelodysplastic syndromes. Curr Treat Options Oncol. 2005;6(3):209-14.

113. Hasle H, Niemeyer CM, Chessells JM, Baumann I, Bennett JM, Kerndrup G, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia. 2003;17(2):277-82.

114. Mandel K, Dror Y, Poon A, Freedman MH. A practical, comprehensive classification for pediatric myelodysplastic syndromes: the CCC system. J Pediatr Hematol Oncol. 2002;24(7):596-605.

115. Occhipinti E, Correa H, Yu L, Craver R. Comparison of two new classifications for pediatric myelodysplastic and myeloproliferative disorders. Pediatric blood & cancer. 2005;44(3):240-4.

116. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet (London, England). 2000;355(9199):165-9.

117. Niemeyer CM, Arico M, Basso G, Biondi A, Cantu Rajnoldi A, Creutzig U, et al. Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. European Working Group on Myelodysplastic Syndromes in Childhood (EWOG-MDS). Blood. 1997;89(10):3534-43.

118. Hasle H, Arico M, Basso G, Biondi A, Cantu Rajnoldi A, Creutzig U, et al. Myelodysplastic syndrome, juvenile myelomonocytic leukemia, and acute myeloid leukemia associated with complete or partial monosomy 7. European Working Group on MDS in Childhood (EWOG-MDS). Leukemia. 1999;13(3):376-85.

119. Woods WG, Barnard DR, Alonzo TA, Buckley JD, Kobrinsky N, Arthur DC, et al. Prospective study of 90 children requiring treatment for juvenile myelomonocytic leukemia or myelodysplastic syndrome: a report from the Children's Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(2):434-40.

120. Freedman MH, Estrov Z, Chan HS. Juvenile chronic myelogenous leukemia. Am J Pediatr Hematol Oncol. 1988;10(3):261-7.

121. Yoshimi A, Bader P, Matthes-Martin S, Stary J, Sedlacek P, Duffner U, et al. Donor leukocyte infusion after hematopoietic stem cell transplantation in patients with juvenile myelomonocytic leukemia. Leukemia. 2005;19(6):971-7.

122. Creutzig U, Bender-Gotze C, Ritter J, Zimmermann M, Stollmann-Gibbels B, Korholz D, et al. The role of intensive AML-specific therapy in treatment of children with RAEB and RAEB-t. Leukemia. 1998;12(5):652-9.

123. Locatelli F, Nollke P, Zecca M, Korthof E, Lanino E, Peters C, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood. 2005;105(1):410-9.

124. Arico M, Biondi A, Pui CH. Juvenile myelomonocytic leukemia. Blood. 1997;90(2):479-88.

125. Bunin N, Saunders F, Leahey A, Doyle J, Calderwood S, Freedman MH. Alternative donor bone marrow transplantation for children with juvenile myelomonocytic leukemia. J Pediatr Hematol Oncol. 1999;21(6):479-85.

126. Smith FO, King R, Nelson G, Wagner JE, Robertson KA, Sanders JE, et al. Unrelated donor bone marrow transplantation for children with juvenile myelomonocytic leukaemia. British journal of haematology. 2002;116(3):716-24.

127. Moskowitz CH, Nimer SD, Zelenetz AD, Trippett T, Hedrick EE, Filippa DA, et al. A 2-step comprehensive high-dose chemoradiotherapy second-line program for relapsed and refractory Hodgkin disease: analysis by intent to treat and development of a prognostic model. Blood. 2001;97(3):616-23.

128. Schellong G, Dorffel W, Claviez A, Korholz D, Mann G, Scheel-Walter HG, et al. Salvage therapy of progressive and recurrent Hodgkin's disease: results from a multicenter study of the pediatric DAL/GPOH-HD study group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(25):6181-9.

129. Nachman JB, Sposto R, Herzog P, Gilchrist GS, Wolden SL, Thomson J, et al. Randomized comparison of low-dose involved-field radiotherapy and no radiotherapy for children with Hodgkin's disease who achieve a complete response to chemotherapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(18):3765-71.

130. Aparicio J, Segura A, Garcera S, Oltra A, Santaballa A, Yuste A, et al. ESHAP is an active regimen for relapsing Hodgkin's disease. Ann Oncol. 1999;10(5):593-5.

131. Bonfante V, Viviani S, Santoro A, Devizzi L, Di Russo A, Zanini M, et al. Ifosfamide and vinorelbine: an active regimen for patients with relapsed or refractory Hodgkin's disease. British journal of haematology. 1998;103(2):533-5.

132. Zinzani PL, Bendandi M, Stefoni V, Albertini P, Gherlinzoni F, Tani M, et al. Value of gemcitabine treatment in heavily pretreated Hodgkin's disease patients. Haematologica. 2000;85(9):926-9.

133. Santoro A, Bredenfeld H, Devizzi L, Tesch H, Bonfante V, Viviani S, et al. Gemcitabine in the treatment of refractory Hodgkin's disease: results of a multicenter phase II study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2000;18(13):2615-9.

134. Peniket AJ, Ruiz de Elvira MC, Taghipour G, Cordonnier C, Gluckman E, de Witte T, et al. An EBMT registry matched study of allogeneic stem cell transplants for lymphoma: allogeneic transplantation is associated with a lower relapse rate but a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transplant. 2003;31(8):667-78.

135. Lieskovsky YE, Donaldson SS, Torres MA, Wong RM, Amylon MD, Link MP, et al. High-dose therapy and autologous hematopoietic stem-cell transplantation for recurrent or refractory pediatric Hodgkin's disease: results and prognostic indices. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2004;22(22):4532-40.

136. Akhtar S, Weshi AE, Rahal M, Khafaga Y, Tbakhi A, Humaidan H, et al. Factors affecting autologous peripheral blood stem cell collection in patients with relapsed or refractory diffuse large cell lymphoma and Hodgkin lymphoma: a single institution result of 168 patients. Leukemia & lymphoma. 2008;49(4):769-78.

137. Cooney JP, Stiff PJ, Toor AA, Parthasarathy M. BEAM allogeneic transplantation for patients with Hodgkin's disease who relapse after autologous transplantation is safe and effective. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2003;9(3):177-82.

138. Claviez A, Klingebiel T, Beyer J, Nurnberger W, Ehninger G, Suttorp M, et al. Allogeneic peripheral blood stem cell transplantation following fludarabine-based conditioning in six children with advanced Hodgkin's disease. Ann Hematol. 2004;83(4):237-41.

139. Sureda A, Schmitz N. Role of allogeneic stem cell transplantation in relapsed or refractory Hodgkin's disease. Ann Oncol. 2002;13 Suppl 1:128-32.

140. Attarbaschi A, Dworzak M, Steiner M, Urban C, Fink FM, Reiter A, et al. Outcome of children with primary resistant or relapsed non-Hodgkin lymphoma and mature B-cell leukemia after intensive first-line treatment: a population-based analysis of the Austrian Cooperative Study Group. Pediatric blood & cancer. 2005;44(1):70-6.

141. Kobrinsky NL, Sposto R, Shah NR, Anderson JR, DeLaat C, Morse M, et al. Outcomes of treatment of children and adolescents with recurrent non-Hodgkin's lymphoma and Hodgkin's disease with dexamethasone, etoposide, cisplatin, cytarabine, and l-asparaginase, maintenance chemotherapy, and transplantation: Children's Cancer Group Study CCG-5912. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(9):2390-6.

142. Attias D, Weitzman S. The efficacy of rituximab in high-grade pediatric B-cell lymphoma/leukemia: a review of available evidence. Curr Opin Pediatr. 2008;20(1):17-22.

143. Saarinen-Pihkala UM, Gustafsson G, Ringden O, Heilmann C, Glomstein A, Lonnerholm G, et al. No disadvantage in outcome of using matched unrelated donors as compared with matched sibling donors for bone marrow transplantation in children with acute lymphoblastic leukemia in second remission. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(14):3406-14.

144. Giebel S, Giorgiani G, Martinetti M, Zecca M, Maccario R, Salvaneschi L, et al. Low incidence of severe acute graft-versus-host disease in children given haematopoietic stem cell transplantation from unrelated donors prospectively matched for HLA class I and II alleles with high-resolution molecular typing. Bone Marrow Transplant. 2003;31(11):987-93.

145. Talano JA, Margolis DA. Recent molecular and cellular advances in pediatric bone marrow transplantation. Pediatr Clin North Am. 2006;53(4):685-98.

146. Balduzzi A, Gooley T, Anasetti C, Sanders JE, Martin PJ, Petersdorf EW, et al. Unrelated donor marrow transplantation in children. Blood. 1995;86(8):3247-56.

147. Davies SM, Wagner JE, Defor T, Blazar BR, Katsanis E, Kersey JH, et al. Unrelated donor bone marrow transplantation for children and adolescents with aplastic anaemia or myelodysplasia. British journal of haematology. 1997;96(4):749-56.

148. Davies SM, Wagner JE, Shu XO, Blazar BR, Katsanis E, Orchard PJ, et al. Unrelated donor bone marrow transplantation for children with acute leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1997;15(2):557-65.

149. Bunin N, Carston M, Wall D, Adams R, Casper J, Kamani N, et al. Unrelated marrow transplantation for children with acute lymphoblastic leukemia in second remission. Blood. 2002;99(9):3151-7.

150. Woolfrey AE, Anasetti C, Storer B, Doney K, Milner LA, Sievers EL, et al. Factors associated with outcome after unrelated marrow transplantation for treatment of acute lymphoblastic leukemia in children. Blood. 2002;99(6):2002-8.

151. Talano JM, Casper JT, Camitta BM, Keever-Taylor CA, Murray KJ, Eapen M, et al. Alternative donor bone marrow transplant for children with Philadelphia chromosome ALL. Bone Marrow Transplant. 2006;37(2):135-41.

152. Duggan P, Booth K, Chaudhry A, Stewart D, Ruether JD, Gluck S, et al. Unrelated donor BMT recipients given pretransplant low-dose antithymocyte globulin have outcomes equivalent to matched sibling BMT: a matched pair analysis. Bone Marrow Transplant. 2002;30(10):681-6.

153. Munoz A, Diaz-Heredia C, Diaz MA, Badell I, Verdeguer A, Martinez A, et al. Allogeneic hemopoietic stem cell transplantation for childhood acute lymphoblastic leukemia in second complete remission-similar outcomes after matched related and unrelated donor transplant: a study of the Spanish Working Party for Blood and Marrow Transplantation in Children (Getmon). Pediatr Hematol Oncol. 2008;25(4):245-59.

154. Uzunel M, Remberger M, Sairafi D, Hassan Z, Mattsson J, Omazic B, et al. Unrelated versus related allogeneic stem cell transplantation after reduced intensity conditioning. Transplantation. 2006;82(7):913-9.

155. Giebel S, Locatelli F, Lamparelli T, Velardi A, Davies S, Frumento G, et al. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. Blood. 2003;102(3):814-9.

156. Maury S, Balere-Appert ML, Chir Z, Boiron JM, Galambrun C, Yakouben K, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica. 2007;92(5):589-96.

157. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007;110(13):4576-83.

158. Loiseau P, Busson M, Balere ML, Dormoy A, Bignon JD, Gagne K, et al. HLA Association with hematopoietic stem cell transplantation outcome: the number of mismatches at HLA-A, -B, -C, -DRB1, or -DQB1 is strongly associated with overall survival. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2007;13(8):965-74.

159. Saber W, Opie S, Rizzo JD, Zhang MJ, Horowitz MM, Schriber J. Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia. Blood. 2012;119(17):3908-16.

160. Finke J, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, et al. Prognostic factors affecting outcome after allogeneic transplantation for hematological malignancies from unrelated donors: results from a randomized trial. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2012;18(11):1716-26.

161. Kernan NA, Bartsch G, Ash RC, Beatty PG, Champlin R, Filipovich A, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. The New England journal of medicine. 1993;328(9):593-602.

162. Champlin RE, Schmitz N, Horowitz MM, Chapuis B, Chopra R, Cornelissen JJ, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). Blood. 2000;95(12):3702-9.

163. Papadopoulos EB, Carabasi MH, Castro-Malaspina H, Childs BH, Mackinnon S, Boulad F, et al. T-cell-depleted allogeneic bone marrow transplantation as postremission therapy for acute myelogenous leukemia: freedom from relapse in the absence of graft-versus-host disease. Blood. 1998;91(3):1083-90.

164. Montero A, Savani BN, Shenoy A, Read EJ, Carter CS, Leitman SF, et al. T-cell depleted peripheral blood stem cell allotransplantation with T-cell add-back for patients with hematological malignancies: effect of chronic GVHD on outcome. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2006;12(12):1318-25.

165. Cornelissen JJ, Lowenberg B. Developments in T-cell depletion of allogeneic stem cell grafts. Curr Opin Hematol. 2000;7(6):348-52.

166. Ottinger HD, Beelen DW, Scheulen B, Schaefer UW, Grosse-Wilde H. Improved immune reconstitution after allotransplantation of peripheral blood stem cells instead of bone marrow. Blood. 1996;88(7):2775-9.

167. Storek J, Witherspoon RP, Maloney DG, Chauncey TR, Storb R. Improved reconstitution of CD4 T cells and B cells but worsened reconstitution of serum IgG levels after allogeneic transplantation of blood stem cells instead of marrow. Blood. 1997;89(10):3891-3.

168. Martinez C, Urbano-Ispizua A, Rozman C, Marin P, Rovira M, Sierra J, et al. Immune reconstitution following allogeneic peripheral blood progenitor cell transplantation: comparison of recipients of positive CD34+ selected grafts with recipients of unmanipulated grafts. Exp Hematol. 1999;27(3):561-8.

169. Behringer D, Bertz H, Schmoor C, Berger C, Dwenger A, Finke J. Quantitative lymphocyte subset reconstitution after allogeneic hematopoietic transplantation from matched related donors with CD34+ selected PBPC grafts unselected PBPC grafts or BM grafts. Bone Marrow Transplant. 1999;24(3):295-302.

170. Chen X, Hale GA, Barfield R, Benaim E, Leung WH, Knowles J, et al. Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical

stem cell graft for paediatric refractory haematological malignancies. British journal of haematology. 2006;135(4):524-32.

171. Bethge WA, Faul C, Bornhäuser M, Stuhler G, Beelen DW, Lang P, et al. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: an update. Blood cells, molecules & diseases. 2008;40(1):13-9.

172. Novitzky N, Thomas V, du Toit C, McDonald A. Reduced-intensity conditioning for severe aplasia using fludarabine and CY followed by infusion of ex vivo T-cell-depleted grafts leads to excellent engraftment and absence of GVHD. Bone Marrow Transplant. 2009;43(10):779-85.

173. Chaudhury S, Auerbach A, Kernan N, Small T, Prockop S, Scaradavou A, et al. Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anaemia. British journal of haematology. 2008;140(6):644-55.

174. Lang P, Handgretinger R. Haploidentical SCT in children: an update and future perspectives. Bone Marrow Transplant. 2008;42(S2):S54-S9.

175. Aversa F, Tabilio A, Terenzi A, Velardi A, Falzetti F, Giannoni C, et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood. 1994;84(11):3948-55.

176. Aversa F, Tabilio A, Velardi A, Cunningham I, Terenzi A, Falzetti F, et al. Treatment of High-Risk Acute Leukemia with T-Cell-Depleted Stem Cells from Related Donors with One Fully Mismatched HLA Haplotype. The New England journal of medicine. 1998;339(17):1186-93.

177. Aversa F, Terenzi A, Felicini R, Tabilio A, Falzetti F, Carotti A, et al. Mismatched T cell-depleted hematopoietic stem cell transplantation for children with high-risk acute leukemia. Bone Marrow Transplant. 1998;22 Suppl(5):S29-32.

178. Aversa F, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S, et al. Full Haplotype-Mismatched Hematopoietic Stem-Cell Transplantation: A Phase II Study in Patients With Acute Leukemia at High Risk of Relapse. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(15):3447-54.

179. Elhasid R, Arush MB, Zaidman I, Leiba R, Barak AB, Postovsky S, et al. Safe and Efficacious Allogeneic Bone Marrow Transplantation for Nonmalignant Disorders Using Partial T Cell Depletion and No Posttransplantation Graft-Versus-Host-Disease Prophylaxis. Biology of Blood and Marrow Transplantation. 2007;13(3):329-38.

180. Castriconi R, Dondero A, Corrias MV, Lanino E, Pende D, Moretta L, et al. Natural killer cell-mediated killing of freshly isolated neuroblastoma cells: critical role of DNAX accessory molecule-1-poliovirus receptor interaction. Cancer research. 2004;64(24):9180-4.

181. Prigione I, Corrias M, Airoldi I, Raffaghello L, Morandi F, Bocca P, et al. Immunogenicity of Human Neuroblastoma. Annals of the New York Academy of Sciences. 2004;1028(Signal Transduction and Communication in Cancer Cells):69-80.

182. Chin T, Toy C, Vandeven C, Cairo MS. Lymphokine-activated killer cytotoxicity in neonatal mononuclear cells: in vitro responses to tumor cell lines from pediatric solid tumors. Pediatric research. 1989;25(2):156-60.

183. Klob S, Bochennek K, Huenecke S, Zimmermann S-Y, KuÁi S, M¸ller T, et al. A novel five-colour flow cytometric assay to determine NK cell cytotoxicity against neuroblastoma and other adherent tumour cells. Journal of Immunological Methods. 2007;325(1-2):140-7.

184. Castriconi R, Dondero A, Cilli M, Ognio E, Pezzolo A, De Giovanni B, et al. Human NK cell infusions prolong survival of metastatic human neuroblastoma-bearing NOD/scid mice. Cancer immunology, immunotherapy. 2007;56(11):1733-42.

185. Osenga KL, Hank JA, Albertini MR, Gan J, Sternberg AG, Eickhoff J, et al. A Phase I Clinical Trial of the hu14.18-IL2 (EMD 273063) as a Treatment for Children with Refractory or Recurrent Neuroblastoma and Melanoma: a Study of the Children's Oncology Group. Clinical cancer research : an official journal of the American Association for Cancer Research. 2006;12(6):1750-9.

186. Capitini CM, Cooper LJ, Egeler RM, Handgretinger R, Locatelli F, Sondel PM, et al. Highlights of the First International "Immunotherapy in Pediatric Oncology: Progress and Challenges" Meeting. J Pediatr Hematol Oncol. 2009;31(4):227-44.

187. Verhoeven DHJ, de Hooge ASK, Mooiman ECK, Santos SJ, ten Dam MM, Gelderblom H, et al. NK cells recognize and lyse Ewing sarcoma cells through NKG2D and DNAM-1 receptor dependent pathways. Molecular immunology. 2008;45(15):3917-25.

188. Wu A, Wiesner S, Xiao J, Ericson K, Chen W, Hall W, et al. Expression of MHC I and NK ligands on human CD133+ glioma cells: possible targets of immunotherapy. Journal of Neuro-Oncology. 2007;83(2):121-31.

189. Multhoff G, Botzler C, Wiesnet M, Eissner G, Issels R. CD3- large granular lymphocytes recognize a heat-inducible immunogenic determinant associated with the 72-kD heat shock protein on human sarcoma cells. Blood. 1995;86(4):1374-82.

190. Nolibé D, Poupon MF. Enhancement of pulmonary metastases induced by decreased lung natural killer cell activity. Journal of the National Cancer Institute. 1986;77(1):99-103.

191. Evans R, Fuller JA, Christianson G, Krupke DM, Troutt AB. IL-15 Mediates Anti-tumor Effects after Cyclophosphamide Injection of Tumor-Bearing Mice and Enhances Adoptive Immunotherapy: The Potential Role of NK Cell Subpopulations. Cellular Immunology. 1997;179(1):66-73.

192. Belova OB, Vinnichuk UD, Shlakhovenko VA, Berezhnaya NM. Efficacy of different immunotherapy approaches toward treatment of doxorubicin-resistant and doxorubicin-sensitive transplantable rhabdomyosarcoma. Eksperimental′naia onkologiia. 2007;29(4):272-6.

193. Bauer M, Reaman GH, Hank JA, Cairo MS, Anderson P, Blazar BR, et al. A phase II trial of human recombinant interleukin-2 administered as a 4-day continuous infusion for children with refractory neuroblastoma, non-Hodgkin's lymphoma, sarcoma, renal cell carcinoma, and malignant melanoma. A Childrens Cancer Group study. Cancer. 1995;75(12):2959-65.

194. Pende D, Marcenaro S, Falco M, Martini S, Bernardo ME, Montagna D, et al. Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and re-definition of inhibitory KIR specificity. Blood. 2008:blood-2008-06-164103.

195. Boyiadzis M, Memon S, Carson J, Allen K, Szczepanski MJ, Vance BA, et al. Up-regulation of NK cell activating receptors following allogeneic hematopoietic stem cell transplantation under a lymphodepleting reduced intensity regimen is associated with elevated IL-15 levels. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2008;14(3):290-300.

196. Murphy WJ, Keller JR, Harrison CL, Young HA, Longo DL. Interleukin-2-activated natural killer cells can support hematopoiesis in vitro and promote marrow engraftment in vivo. Blood. 1992;80(3):670-7.

197. Gewirtz AM, Xu WY, Mangan KF. Role of natural killer cells, in comparison with T lymphocytes and monocytes, in the regulation of normal human megakaryocytopoiesis in vitro. Journal of immunology (Baltimore, Md : 1950). 1987;139(9):2915-24.

198. Murphy WJ, Longo DL. Hematopoietic growth-promoting effects of natural killer cells in mice in response to in vivo administration of human interleukin-2. J Natl Cancer Inst. 1992;84(15):1195-200.

199. Murphy WJ, Raziuddin A, Mason L, Kumar V, Bennett M, Longo DL. NK cell subsets in the regulation of murine hematopoiesis. I. 5E6+ NK cells promote hematopoietic growth in H-2d strain mice. Journal of immunology (Baltimore, Md : 1950). 1995;155(6):2911-7.

200. Hsu KC, Keever-Taylor CA, Wilton A, Pinto C, Heller G, Arkun K, et al. Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood. 2005;105(12):4878-84.

201. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105(8):3051-7.

202. Passweg JR, Stern M, Koehl U, Uharek L, Tichelli A. Use of natural killer cells in hematopoetic stem cell transplantation. Bone Marrow Transplant. 2005;35(7):637-43.

203. Passweg JR, Tichelli A, Meyer-Monard S, Heim D, Stern M, Kuhne T, et al. Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. Leukemia. 2004;18(11):1835-8.

204. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science (New York, NY). 2002;295(5562):2097-100.

205. Murphy W, Koh C, Raziuddin A, Bennett M, Longo D. Immunobiology of natural killer cells and bone marrow transplantation: merging of basic and preclinical studies. Immunological reviews. 2001;181(1):279-89.

206. Asai O, Longo DL, Tian ZG, Hornung RL, Taub DD, Ruscetti FW, et al. Suppression of graft-versus-host disease and amplification of graft-versus-tumor effects by activated natural

killer cells after allogeneic bone marrow transplantation. The Journal of clinical investigation. 1998;101(9):1835-42.

207. Lang P. Co-Transfusion of Donor NK Cells in Haploidentical Stem Cell Transplantation. Blood. 2008;112(ASH Annual Meeting Abstracts ):Abstract 2907.

208. Rizzieri D. Safety Trial of NK Cell Enhanced Donor Lymphocyte Infusions from a 3-5/6 HLA Matched Family Member Following Nonmyeloablative Allogeneic Stem Cell Transplantation. Blood. 2008;112(ASH Annual Meeting Abstracts):Abstract 342.

209. Siegler U, Kalberer CP, Nowbakht P, Sendelov S, Meyer-Monard S, Wodnar-Filipowicz A. Activated natural killer cells from patients with acute myeloid leukemia are cytotoxic against autologous leukemic blasts in NOD//SCID mice. Leukemia. 2005;19(12):2215-22.

210. Isgro A. Donor's NK Cells May Influence the Engraftment in Pediatrics Patients after T-Cell Depleted Haploidentical Stem Cell Transplant for Thalassemia. Blood. 2008;112(ASH Annual Meeting Abstracts):Abstract 4595.

211. Imai C, Iwamoto S, Campana D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. Blood. 2005;106(1):376-83.

212. Sanders JE, Thomas ED, Buckner CD, Doney K. Marrow transplantation for children with acute lymphoblastic leukemia in second remission. Blood. 1987;70(1):324-6.

213. Wiener LS, Steffen-Smith E, Fry T, Wayne AS. Hematopoietic stem cell donation in children: a review of the sibling donor experience. J Psychosoc Oncol. 2007;25(1):45-66.

# 11 APPENDICES

## 11.1 APPENDIX 1: PERFORMANCE STATUS

| **PERFORMANCE STATUS CRITERIA** *Karnofsky and Lansky performance scores are intended to be multiples of 10.* | | | | | |
|---|---|---|---|---|---|
| **ECOG (Zubrod)** | | **Karnofsky** | | **Lansky** | |
| **Score** | **Description** | **Score** | **Description** | **Score** | **Description** |
| 0 | Fully active, able to carry on all pre-disease performance without restriction. | 100% | Normal, no complaints, no evidence of disease. | 100% | Fully active, normal. |
| | | 90% | Able to carry on normal activity; minor sighs of symptoms of disease. | 90% | Minor restrictions in physically strenuous activity. |
| 1 | Restricted in physically strenuous activity but ambulatory, able to carry out light or sedentary work, e.g., light housework, office work. | 80% | Able to carry on normal activity with effort; some signs or symptoms of disease. | 80% | Active, but tires more quickly. |
| | | 70% | Cares for self, unable to carry on normal activity or do active work. | 70% | Both greater restriction of, and less time spent in, play activities. |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours. | 60% | Requires occasional assistance but is able to care for most of own needs. | 60% | Up and around, but minimal active play; keeps busy with quieter activities. |
| | | 50% | Requires considerable assistance and frequent medical care. | 50% | Gets dressed, but lies around much of the day; no active play; able to participate in quiet play and activities. |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours | 40% | Disabled; requires special care and assistance. | 40% | Mostly in bed; participates in quiet activities. |

| | | | | | |
|---|---|---|---|---|---|
| | | 30% | Severely disabled; hospitalization indicated, although death not imminent. | 30% | In bed; needs assistance even for quiet play. |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to a bed or chair | 20% | Very ill; hospitalization necessary; active supportive treatment required. | 20% | Often sleeping; play entirely limited to very passive activities. |
| | | 10% | Moribund, fatal process progressing rapidly | 10% | No play; does not get out of bed |
| 5 | Dead | 0% | Patient expired | 0% | Unresponsive; Dead |

## 11.2 APPENDIX 2: REQUIRED STUDY EVALUATIONS

### 11.2.1 Appendix 2 A: Patient

Required Study Evaluations PATIENT

| Treatment Phase | Eligibility | On-Study | Induction | Pre- | Pre- | BMT prep | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | | | D 1 - 21 | Induction #2&3 | BMT | -6 to -1 | 0 | 14 | 28±7 days | 60±21 days | 100±30 days | 180±30 days | 6-12 MO±3 MO | 12-60 MO±3 MO | Off-Treatment |
| History & Physical - vital signs | X | X | weekly | X | X | daily | daily until discharge | | 2/week | 2/week | 2/week | monthly | monthly | monthly | X |
| Pathology Review | X | | | | | | | | | | | | | | |
| HLA Typing | X | | | | | | | | | | | | | | |
| Type/Screen | X | | | | | q 4 days | q 4 days until d/c | | X* | X* | X* | X* | X* | | |
| DAT* | | | | | | | daily on days 6-10* | | | | | | | | |
| RBC Phenotype/Isohem Titer* | | X | | | | | | | X* | X* | X* | X* | X* | | |
| Urinalysis | X | | | X each cycle | X | daily | daily | until d/c | | | | | | | |
| CBC | X | X | 2x/week | X | X | daily | daily until d/c | | weekly | weekly | weekly | monthly | monthly | X* | X |
| Reticulocyte count | | | | | | weekly | weekly until d/c | | weekly | weekly | weekly | X* | X* | X* | |
| PT, PTT, Lupus anti-coagulant | X | | | | | | | | | | | | | | |
| Chemistry | X | X | 2x/week | X | X | QD | daily until d/c | | X* | X* | X* | X* | X* | X* | X |
| Iron, Transferrin, Ferritin | X | | | | | | | | monthly | monthly | monthly | | | | |
| Urine CrCl, Ca/Cr* | X | | | | | | | | | | | | | | |
| Urine Pregnancy* | X | | X | X | | | | | | | | | | | |
| EKG | X | | | | X | | | | | | | X | * | * | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Echo or MUGA | X | | | | X | | | | | | | X | * | * | |
| PFT/DLCO | X | | | | | | | | | | GVHD | X | * | * | |
| Disease and GVHD Staging Evaluation | X | | | X | X | | | | X | | X | X | q 3mos | q 3mos | |
| Bone Marrow* | X | <14 days | | X* | X* | | | | X* | | X* | X* | X* | X* | |
| CSF* | X | | | | | | | | | | | | | | |
| DPA (>18 years old only) | X | | | | | | | | | | | | | | |
| Endocrine function tests* | | | | | | | | | | | | | * | * | * |
| Bcr-abl PCR blood (28-35d post NK) | | | | | | | | | | | | | | | |
| Consults | | | | | | | | | | | | | | | |
| Social Work | X | | | | | | | | | | | | | | |
| Dental | | X | | | | | | | | | | | | | |
| ID Monitoring & Prophylaxis | | | | | | | | | | | | | | | |
| Infectious Disease Markers | X | | | | | | | | | | | | | | |
| Viral Serology (HSV, VZV, EBV, Toxo) | X | | | | | | | | | | | | | | |
| PPD/Control* | X | | | | | | | | | | | | | | |
| Fluconazole | | | Start, hold during vincristine | | | | | | | | | | | | |
| PCP Prophylaxis | | | Start | | | | | | | | | | | | |
| Viral monitoring and prophylaxis | | | | | Start | | | | | | | | | | |
| Viral Monitoring (CMV, EBV, HHV6, adeno virus) | | | | | | | weekly | weekly | weekly | weekly | weekly | X* | X* | X* | X* |
| IgG Level | | X | | | | | weekly | weekly | weekly | weekly | weekly | X* | X* | X* | |
| Post-vaccine antibody titers | | | | | | | | | | | | | X* | X* | X* |

| Research Studies | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum Storage | | X | | | | | | | | | | | | | |
| Immune surveillance (TBNK or flow) | | X | | X | X | | X | | | | | | | | |
| Chimerism - Peripheral Blood | X | | | | | | | X | X | X | X | * | * | * | * |
| Chimerism - Bone Marrow | | | | | | | | | * | * | * | * | * | | |
| KIR/HLA Genotyping | Anytime prior to HSCT | | | | | | | | | | X | | | | |
| Immune Reconstitution | | X | | | | | | | X | X | X | X | q 3 mo | | |
| Lymphocyte Proliferation | | | | | | | | | X | X | X | X | q 6 mo | | |

** as clinically indicated*

11.2.2 Appendix 2 B: Donor

| Treatment Phase | Eligibility | On-Study | Pre- | Pre- | | Post- | 1-2 week |
|---|---|---|---|---|---|---|---|
| Day | | | CVL | Collection | Collection | Collection | F/U |
| History & Physical | X | | <72 hrs | | | | X |
| DTM Screen | X | | | X | | | |
| HLA Typing | X | | | | | | |
| Type/Screen | X | | | | | | |
| Red Cell Phenotype* | | X* | | | | | |
| Isohemmaglutinin Titer* | | X* | | | | | |
| Urinalysis | X | | | | | | |
| CBC | X | | <72 hrs | X | | X | |
| PT, PTT | X | | <72 hrs | | | | |
| Chemistry | X | | | | | | |
| Infectious Disease Markers | X | | | <30 days | X | | |
| Viral Serology (EBV) | X | | | | | | |
| CD34 Count | | | | | X | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Urine Pregnancy* | X* | | | | | | |
| Consults | | | | | | | |
| Social Work | X | | | | | | |
| Child Psychology/Psychiatry | X* | | | | | | |
| Research Studies | | | | | | | |
| Serum Storage | | X | | | | | |
| Chimerism (STR profile) | | | | X | | | |
| KIR testing | | | | X | | | |

^ Avoid antecubital phlebotomy if possible.

* *as clinically indicated*

## 11.3 APPENDIX 3: SCREENING AND BASELINE EVALUATION

| | RECIPIENT INFORMATION | | | DONOR INFORMATION | | |
|---|---|---|---|---|---|---|
| Name | | | | | | |
| MR# | | | | | | |
| Diagnosis | | | | Stem Cell Collection Dose | | |
| DOB (Age) | | | | | | |
| Wt(kg)/Ht(cm)/BSA(m2) | | | | | | |
| Sex | | | | | | |
| Test | Date | Results | Comments/Inititals | Date | Results | Comments/Inititals |
| History & Physical | | | | | | |
| Immunization Records | | | | | | |
| Donor Questionairre | | | | | | |
| Diagnostic Pathology | | | | | | |
| Blood Type | | | | | | |
| Antibody Screen | | | | | | |
| RBC Phenotype* | | | | | | |
| Isohemmaglutinin Titer* | | | | | | |
| Urinalysis | | | | | | |
| CBC | | | | | | |
| PT, PTT, Lupus anticoagulant | | | | | | |
| Chemistry | | | | | | |
| Urine CrCl | | | | | | |
| Urine Ca/Cr | | | | | | |
| Iron, Transferrin, Ferritin | | | | | | |
| HLA: initial and confirmatory | | | | | | |
| Chimerism (STR Profile) | | | | | | |
| HIV-1/2 | | | | | | |
| NAT | | | | | | |
| HTLV-I/II | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| RPR | | | | | | |
| HB S Ag | | | | | | |
| HB core Ab | | | | | | |
| HCV | | | | | | |
| CMV IgG/IgM | | | | | | |
| Toxo IgG | | | | | | |
| HSV IgG | | | | | | |
| Varicella IgG | | | | | | |
| EBV Panel | | | | | | |
| PPD/Control* | | | | | | |
| Fanconi Anemia test (MDS only) | | | | | | |
| Urine Pregnancy* | | | | | | |
| EKG | | | | | | |
| Echo (SF) or MUGA (EF) | | | | | | |
| | | | | | | |
| PFT/DLCO-VAcorr | | | | | | |
| Staging: | | | | | | |
| PET/CT | | | | | | |
| BM | | | | | | |
| CSF | | | | | | |
| Consults | | | | | | |
| Social Work | | | | | | |
| Dental | | | | | | |
| Child Psych (minors only) | | | | | | |
| CVL / Consent | | | | | | |
| Informed Consent | | | | | | |
| CIBMTR Consent | | | | | | |
| IDMs-BMT <30 days of collection | | | | | | |

*Workup reviewed and confirmed complete by:*

Attending Physician date

## 11.4 APPENDIX 4: SUMMARY OF RESEARCH BLOODS*

| | | SAMPLE ACQUISITION TIME POINTS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Study** | **Laboratory Location** | **Pre-Study** | **Induction** | **Pre-BMT Prep** | **Post-BMT** | **Volume** | **Tube** | **Notes** |
| **Donor & Recipient Serum Storage** | NCI-Frederick | Donor and Recipient | | | | 10 ml | Red top | Sample refrigerator on patient unit<br>^Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up |
| **Immuno-Phenotyping** (Lymphocyte Phenotyping-TBNK panel or NK Subset)** | Clinical Path Flow Cytometry 10/2C410<br>6-4879 | On-Study<br>(Lymphocyte Phenotyping-NK Subset) | Prior to Each Cycle | Day –7, 0 if ALC >200 | 1st day ALC >200; 24 hours +/- 24 hours post NK#1 and #2 infusion. Day 28( ± 7), 60( ± 21), 100( ± 30), and every 3 months ( ± 1 mo) until 2 yrs | 5 ml | Purple top<br>(EDTA) | Schedule before sending<br>X 64879 |
| **Chimerism (STR Profile)** | Dr. Kurlander Hematology Lab 435-6399 | Eligibility | | | Day 14^, 28^+, 60, 100+, then PRN; both whole blood and WBC STR chimerism. | 10 ml ^20 ml +1-2 ml marrow | Blood: yellow top (ACD) Marrow: heparinized syringe | ^20 ml for cell sorting (until 100% donor); + blood and marrow. Schedule with lab X 56399 |
| **Cytokine Levels**<br>**(IL-7/IL-15)** | 10/ C210<br>2-1298 | On-Study | | Day –7, Day 0 | Day prior to or morning of EACH NK infusion, the Day 1, 2, 7, &14 ( ± 48 hr each )after each NK cell infusion, then D 60( ± 21), D100( ± 30), and then q 3 mos ( ± 1 mo) for 2 yrs | 4 ml | red top | Sample refrigerator on patient unit<br>^Courier to NCI-Frederick repository |
| **TH1/TH2 cytokine profile** | SAIC-Frederick<br>(301) 846-1707 | On-Study | | Day –7, Day 0 | Day prior to or morning of EACH NK infusion, the Day 1, 2, 7, &14 ( ± 48 hr each ) after each NK cell infusion, then D 60 ( ± 21), D100 ( ± 30), and then q 3 mos ( ± 1 mo) for 2 yrs | 4 ml | red top | Sample refrigerator on patient unit<br>^Courier to NCI-Frederick repository |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **KIR genotyping** | Delbrook RN<br>1W-3750 | | Recipient -Prior to prep<br><br>Donor – prior to PBSC collection | | Day 100 ± 30d | 1 -2 mL | Blood: yellow top (ACD) tube OR<br>3 buccal swabs | Sample stored and sent at room temp. Deidentified prior to sending and label 11-C-0073–subject ID#. Send to Lihua Hou/Carolyn Hurley at C. W. Bill Young Marrow Program, Georgetown University, 3rd Floor, 11333 Woodglen Drive, Rockville, MD 20852, 301-998-8900. |
| **Lymphocyte Proliferation** | NCI-Frederick | | | | Day 28 ( ± 7), 60 ( ± 21), 100( ± 30), 180 ( ± 30), and every 6 months ( ± 30d) until 2 yrs | 20 ml | green top (sodium heparin) | Sample refrigerator on patient unit<br>^Courier to NCI-Frederick repository |
| **Blasts (Bone Marrow or Blood)** | NCI-Frederick | | | | Relapse | 10ml marrow; 20ml blood | Preservative-free heparin | Sample refrigerator on patient unit<br>^Courier to NCI-Frederick repository |
| **Single-Pass Apheresis** | DTM | | | | Relapse: post-subsequent remission | | | ^Courier to NCI-Frederick repository |
| **Donor Lymphocytes** | DTM | | | | Only if DLI collection | | | ^Courier to NCI-Frederick repository |
| **BCR abl PCR; p210** | Kurlander Lab<br>435-6399 | | | | 28-35 days post final NK infusion | 10 ml | Blood: yellow ACD tube- | Sent as clinical lab (must be arranged with Dr. Roger Kurlander lab). |

***In the event that blood draws are limited due to patient size, research studies are listed in order of priority.**

**^Courier to NCI-Frederick repository 301-846-5893 to schedule pick-up**

****May be substituted for TBNK panel if lymphocyte phenotyping cannot be scheduled.**

## 11.5 APPENDIX 5: ACUTE GVHD

Does the patient have Acute GVHD? ❒No / ❒Yes → Date of the first diagnosis of aGVHD? __/__/____

Did the patient have an aGVHD flare since last exam? ❒No ❒Yes → Date: __/__/____

*Select the appropriate stage, certainty of the system involved and indicate whether a biopsy was completed.*

| **Skin** | | **Certainty** | **Comments** | **Biopsy** |
|---|---|---|---|---|
| **Rash** | **Stage** | | | **Y/N** |
| ❒ Rash not attributable to GVHD | **N/A** | | | |
| ❒ No rash | **0** | **❒Unlikely ❒Possible ❒ Probable ❒ Certain** | | ❒No ❒ Yes ❒ Pending |
| ❒ <25% BSA | **1** | | | |
| ❒ 25-50% BSA | **2** | | | |
| ❒ >50% | **3** | | | |
| ❒ bullae and desquamation | **4** | | | |

| **Liver** | | **Certainty** | **Comments** | **Biopsy** |
|---|---|---|---|---|
| **Bilirubin** | **Stage** | | | **Y/N** |
| ❒ Bilirubin not attributable to GVHD | **N/A** | | | |
| ❒ <2.0 mg/dl | **0** | **❒Unlikely ❒Possible ❒ Probable ❒ Certain** | | ❒No ❒ Yes ❒ Pendin |
| ❒ 2-3.0 mg/dl | **1** | | | |
| ❒ 3.1-6.0 mg/dl (3.1-8.0 Glucksberg) | **2** | | | |
| ❒ 6.1-15 mg/dl (8.1-15.0 Glucksberg) | **3** | | | |
| ❒ >15 mg/dl | **4** | | | |

| **Upper GI** | **GI Tract** | | **Certainty** | **Comments** | **Biopsy** |
|---|---|---|---|---|---|
| **Persistent N/V** | **Diarrhea ml/day (peds ml/m2/day)** | **Stage** | | | **Y/N** |
| | ❒ Diarrhea not attributable to GVHD | **N/A** | | | |
| ❒ No (0) | ❒ No diarrhea | **0** | **❒Unlikely ❒Possible ❒ Probable ❒ Certain** | | ❒No ❒Yes ❒Pending |

| | | |
|---|---|---|
| ❒ Yes (1) | ❒ 500-1,000 (< 280) | **1** |
| | ❒ 1,000-1,500 (280-555) | **2** |
| | ❒ > 1,500 (555-833) | **3** |
| | ❒ severe abdominal pain (w/, w/o ileus) | **4** |

*Grade* Modified Glucksberg Criteria*

| | Skin | Liver | GI |
|---|---|---|---|
| **1** | 1-2 | 0 | 0 |
| **2** | 1-3 | 1 | 1 |
| **$2_o$** | 0 | 1 | 1 |
| **$2_s$** | 4 | 0 | 0 |
| **3** | 2-4 | 2-4 *&/or* | 2-4 (1 only >2) |
| **4** | 3-4 | 2-4 | 2-4 |

*Grade* IBMTR*

| | Skin (max stage) | Liver (max stage) | GI (max stage) |
|---|---|---|---|
| A | 1 | 0 | 0 |
| B | 2 or | 1-2 or | 1-2 |
| C | 3 or | 3 or | 3 |
| D | 4 or | 4 or | 4 |

| | Modified Glucksberg Criteria | CIBMTR Criteria | Date |
|---|---|---|---|
| Current aGVHD Grade: | | | |
| Maximum aGVHD Grade | | | |

What was the max stage in the 1st 100 days per organ:

Skin:____(0-4), Lower GI:____(0-4), Upper GI (i.e. nausea/vomiting):_____(0-1), Liver_____(0-4)

Other organ system involvement: ❒No / ❒Yes → ❒ Lung → biopsy proven: ❒No /❒Yes

❒ Other _____

## 11.6 APPENDIX 6: CHRONIC GVHD EXAM AND GRADING, CLINICIAN ASSESSMENT FORM

Were Functional Performance tests completed? ❒No ❒Yes *(Must be greater than 4 years old)*

Select the tests performed:

❒ Walk Time: Number of laps: _____ (x 50 feet) + final partial lap: _____ feet = _____ feet walked in 2 minutes

**Total Distance Walked in 2 Minutes:**

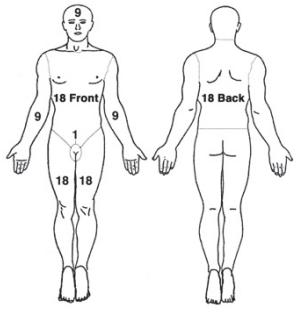
❒ Grip Strength:

**Grip Strength** (Dominant Hand) _____

❒ **Range of Motion:**

❒ Not performed ❒ See Physical Therapy Report

**Skin:** Does the patient have skin changes? ❒No ❒Yes → Check and score all findings that apply.

| Skin Component | Findings | Scoring |
|---|---|---|
|  | ❒ Erythematous rash of any sort | ____% BSA (max 100%) |
| | ❒ Moveable sclerosis | ____ % BSA (max 100%) |
| | ❒ Non-moveable sclerosis (hidebound/non-pinchable) or subcutaneous sclerosis/fasciitis | ____% BSA (max 100%) |
| | ❒ Ulcer(s): select the largest ulcerative lesion, and measure its largest dimension in cm and mark location of ulcer | Location: _____<br>Largest dimension: _____cm |

**Eyes:** Does the patient have decreased tear production? ❒No ❒Yes

Schirmer's Tear Test completed? ❒No ❒Yes → ❒ With anesthesia ❒ Without anesthesia

| | | |
|---|---|---|
| Bilateral Schirmer's Tear Test<br>(without anesthesia) > 9 years or older | Right Eye: _____ mm of wetting | Left Eye: _____ mm of wetting |

**Lungs:** Were Pulmonary Function Tests completed? ❒No ❒Yes

| | | |
|---|---|---|
| **Lungs**<br>• Bronchiolitis Obliterans<br>• Pulmonary Function Tests with Diffusing Capacity | FEV-1<br><br>% Predicted | Single Breath DLCO (adjusted for Hg)<br><br>% Predicted |

**Gastrointestinal:**

| | |
|---|---|
| **Upper GI**<br>• Early satiety OR<br>• Anorexia OR<br>• Nausea/Vomiting<br>**Score:** | 0= no symptoms<br>1=mild, occasional symptoms, with little reduction in oral intake during the past week<br>2=moderate, intermittent symptoms, with some reduction in oral intake during the past week<br>3=more severe or persistent symptoms throughout the day, with marked reduction in oral intake, on almost every day of the past week |
| **GI/Esophageal**<br>• Dysphagia OR<br>• Odynophagia<br>**Score:** | 0= no esophageal symptoms<br>1=Occasional dysphagia or odynophagia with solid food or pills during the past week<br>2=Intermittent dysphagia or odynophagia with solid foods or pills, but not for liquids or soft foods, during the past week<br>3=Dysphagia or odynophagia for almost all oral intake, on almost every day of the past week |
| **Lower GI**<br>• Diarrhea<br>**Score:** | 0= no loose or liquid stools during the past week<br>1= occasional loose or liquid stools, on some days during the past week<br>2=intermittent loose or liquid stools throughout the day, on almost every day of the past week, **without requiring** intervention to prevent or correct volume depletion<br>3=voluminous diarrhea on almost every day of the past week, **requiring** intervention to prevent or correct volume depletion |

**Mucous Membrane:** Does the patient have mucosal changes? ❒No ❒Yes

| **Mucosal change** | No cGvHD | | Mild | | Moderate | | Severe | |
|---|---|---|---|---|---|---|---|---|
| **Erythema** | None | 0 | Mild or moderate erythema (<25%) | 1 | Moderate (≥25%) or Severe erythema (<25%) | 2 | Severe erythema (≥25%) | 3 |
| **Lichenoid** | None | 0 | Hyperkeratotic changes(<25%) | 1 | Hyperkeratotic changes(25-50%) | 2 | Hyperkeratotic changes (>50%) | 3 |
| **Ulcers** | None | 0 | None | 0 | Ulcers involving (≤20%) | 3 | Severe ulcerations (>20%) | 6 |
| **Mucoceles*** | None | 0 | 1-5 mucoceles | 1 | 6-10 scattered mucoceles | 2 | Over 10 mucoceles | 3 |

| | | | |
|---|---|---|---|
| *Mucoceles scored for lower labial and soft palate only | | **Total mucosal changes** | |

**Genital:** Does the patient have involvement of the genital involvement? ❒No ❒Yes

❒ Erythema ❒ Lichen planus-like features ❒ Erosions/fissures ❒ Ulcers

Female patients only: ❒ Agglutination of the clitoral hood ❒ Vulvar sclerosis ❒ Vestibular tenderness ❒ Vaginal scarring/stenosis

**Health Care Provider Global Ratings:**

| | | |
|---|---|---|
| **In your opinion, do you think that this patient's chronic GvHD is mild, moderate or severe?**<br>❒ 0=none<br>❒ 1= mild<br>❒ 2=moderate<br>❒ 3=severe | **Where would you rate the severity of this patient's chronic GvHD symptoms on the following scale, where 0 is cGVHD symptoms that are not at all severe and 10 is the most severe cGVHD symptoms possible:**<br>0 1 2 3 4 5 6 7 8 9 10<br>**Not at all** **Most severe Symptoms Possible**<br>**Provider rating:** _____ | **Over the past <u>month</u> would you say that this patient's cGvHD is**<br>❒ +3= Very much better<br>❒ +2= Moderately better<br>❒ +1= A little better<br>❒ 0= About the same<br>❒ -1=A little worse<br>❒ -2=Moderately worse<br>❒ -3=Very much worse |

**Appendix 6 (continued): Chronic GVHD Exam and Grading**

Does the patient have cGVHD? → ❒No ❒Yes

First diagnosis of cGVHD? ❒Yes ❒No → Date of first cGVHD diagnosis __/__/__

Onset of 1st diagnosis of chronic GVHD was:

- ❒ Progressive (acute GVHD progressed directly to chronic GVHD)
- ❒ Interrupted (acute GVHD resolved, then chronic GVHD developed)
- ❒ De novo (acute GVHD never developed)

Diagnosis was based on: ❒ histologic evidence / biopsy proven ❒ clinical evidence ❒ both ❒ unknown

Did cGVHD flare since last visit? ❒ N/A ❒No ❒Yes → Date: __/__/__

**Maximum grade of chronic GVHD (Seattle Criteria):**

❒ Limited - localized skin involvement and/or hepatic dysfunction due to cGVHD

❒ Extensive - one or more of the following:

– generalized skin involvement; or,
– liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or,
– involvement of eye: Schirmer's test with < 5 mm wetting; or
– involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or
– involvement of any other target organ

**Overall severity of chronic GVHD (NIH Consensus Criteria):**

❒ Mild – signs and symptoms do not interfere substantially with function and do not progress once appropriately treated with local therapy or standard systemic therapy (corticosteroids and/or cyclosporine or FK 506)

❒ Moderate– signs and symptoms interfere somewhat with function despite appropriate therapy or are progressive through first line systemic therapy (corticosteroids and/or cyclosporine or FK 506)

❒ Severe – signs and symptoms limit function substantially despite appropriate therapy or are progressive through second line therapy

## 11.7 Appendix 7: Chronic GVHD, NIH Staging Form

| NIH cGVHD Score | SCORE 0 | SCORE 1 | SCORE 2 | SCORE 3 |
|---|---|---|---|---|
| **Skin**<br>*Clinical features:*<br>☐ Maculopapular rash<br>☐ Lichen planus-like features<br>☐ Papulosquamous lesions or ichthyosis<br>☐ Hyperpigmentation<br>☐ Hypopigmentation<br>☐ Keratosis pilaris<br>☐ Erythema<br>☐ Erythroderma<br>☐ Poikiloderma<br>☐ Sclerotic features<br>☐ Pruritus<br>☐ Hair involvement<br>☐ Nail involvement<br>**% BSA involved** ☐ | ☐ No Symptoms | ☐ <18% BSA with disease signs but **NO** sclerotic features | ☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch) | ☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus |
| **Mouth** | ☐ No symptoms | ☐ Mild symptoms with disease signs but not limiting oral intake significantly | ☐ Moderate symptoms with disease signs **with** partial limitation of oral intake | ☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake |
| **Eyes**<br>Mean tear test (mm):<br>☐ >10<br>☐ 6-10<br>☐ ≤5<br>☐ Not done | ☐ No symptoms | ☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic keratoconjunctivitis sicca | ☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment | ☐ Severe dry eye symptoms significantly affecting ADL (eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca |

| | | | | |
|---|---|---|---|---|
| **GI TRACT** ☐ | ☐ No symptoms | ☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%) | ☐ Symptoms associated with mild to moderate weight loss (5-15%) | ☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation |
| **LIVER** ☐ | ☐ Normal LFT | ☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN | ☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN | ☐ Bilirubin or enzymes > 5 x ULN |

| NIH cGVHD Score | SCORE 0 | SCORE 1 | SCORE 2 | SCORE 3 |
|---|---|---|---|---|
| **Lungs*** <br> **FEV1** ☐ <br> **DLCO** ☐ | ☐ No symptoms <br> ☐ **FEV1 > 80% OR LFS=2** | ☐ Mild symptoms (shortness of breath after climbing one flight of steps) <br> ☐ **FEV1 60-79% OR LFS 3-5** | ☐ Moderate symptoms (shortness of breath after walking on flat ground) <br> ☐ **FEV1 40-59% OR LFS 6-9** | ☐ Severe symptoms (shortness of breath at rest; requiring 02) <br> ☐ **FEV1 <39% OR LFS 10-12** |
| **Joints and Fascia** ☐ | ☐ **No symptoms** | ☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) AND not affecting ADL | ☐ Tightness of arms or legs OR joint contractures, erythema due to fasciitis, moderate decrease ROM AND mild to moderate limitation of ADL | ☐ Contractures WITH significant decrease of ROM AND significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.) |
| **Genital Tract** ☐ | ☐ No symptoms | ☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam | ☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam | ☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum |

## 11.8 APPENDIX 8: LEUKEMIA/LYMPHOMA RESPONSE CRITERIA

*Bone Marrow Classification*

| | **%** blasts (at least 200 cells counted) |
|---|---|
| M1 | <5% |
| M2 | 5 - 25% |
| M3 | > 25% |

*CNS Classification*

| | CSF Cytospin |
|---|---|
| CNS 1 | 0 blasts on cytopsin |
| CNS 2 | White cell count < 5/µl, blasts on cytopsin |
| CNS 3 | White cell count ≥ 5/µl; blasts on cytospin |

**Acute Leukemia** (Adapted from Cheson BD, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 2003;21:4642-4649)

Complete Response (CR)

- M1 marrow, absence of peripheral blasts, absence of extramedullary sites of disease, peripheral blood neutrophil count ≥ 1,000/µL and platelet count ≥ 100,000/µL.
- Morphologic CR with incomplete blood count recovery (CRi): Above CR criteria without specified blood counts.
- Cytogenetic CR (CRc): In addition to above CR criteria, reversion to normal karyotype for those with previously detected cytogenetic abnormality.
- Molecular CR (CRm): In addition to above CRc criteria, normalization of previously detected molecular cytogenetic abnormality.

Partial Response (PR)

- M2 marrow and a decrease in the percentage of marrow blasts and absolute peripheral blast count by at least 50%, absence of extramedullary sites of disease, peripheral blood neutrophil count ≥ 1,000/µL and platelet count ≥ 100,000/µL.

Stable Disease (SD)

- No change in marrow classification (i.e., M status) and less than a 50% decrease to less than a 50% increase in absolute peripheral blast count or extent of extramedullary disease

Progressive Disease (PD)

- Worse marrow classification (i.e., M status) with at least a 50% increase in the percentage of marrow blasts.

Or

- No change in marrow classification (i.e., M status), but a 50% or greater increase in absolute peripheral blast count or extent of extramedullary disease

## Chronic Myelogenous Leukemia

| | **Hematologic** | **Cytogenetic** | **Molecular** |
|---|---|---|---|
| CR | Normal CBC, no splenomegaly | 0% Ph+ cells | Undetectable *bcr/abl* by PCR |
| PR | $\geq$ 50% decrease in peripheral WBC and WBC < 20,000; persistent splenomegaly | $\geq$ 50% decrease in Ph+ cells | Detectable *bcr/abl* by PCR |
| SD | < 50% decrease in peripheral WBC | No new cytogenetic abnormalities | Detectable *bcr/abl* by PCR |
| PD | Worse CBC or splenomegaly | New cytogenetic abnormalities | Detectable *bcr/abl* by PCR |

**Lymphoma** (Adapted from: Cheson BD, Horning SH, Coiffier B, et al: Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. J Clin Oncol 1999;17: 1244-53)

| **Response** | **Physical Exam** | **Lymph Nodes** | **Lymph Node Masses** | **Bone Marrow** |
|---|---|---|---|---|
| CR | Normal | Normal | Normal | Normal |
| CRu | Normal | Normal | Normal | Indeterminate |
| | Normal | Normal | > 75% decrease x 3 mos | Normal or indeterminate |
| PR | Normal | Normal | Normal | Positive |
| | Normal | $\geq$ 50% decrease | $\geq$ 50% decrease | Irrelevant |
| | Decrease in liver/spleen | $\geq$ 50% decrease | $\geq$ 50% decrease | Irrelevant |

| | | | | |
|---|---|---|---|---|
| PD | Enlarging liver/spleen, new sites | New or increased | New or increased | Reappearance |

Complete Remission (CR): Disappearance or reduction to normal size of all measurable and evaluable disease (lymph nodes ≤1.5 cm or nodal masses decreased by > 75% in the sum of the products of the greatest diameters lasting for ≥ 3 months consistent with scar), negative bone marrow, non-palpable spleen.

Partial Response (PR): Reduction in tumor burden by at least 50%. The sum of the products of perpendicular measurements of all (or a representative number of) masses judged to be malignant must decrease by at least 50%. None of these masses may show an increase >25% in size and no new malignant lesions should appear. Sites of disease which cannot be quantitated, such as bone marrow aspirates or lesions showing wide fluctuations prior to treatment, cannot be used to determine a PR. While defined splenic nodules can be considered measurable for the purpose of determining a PR, total spleen size cannot, although a PR is ruled out if the spleen size increases > 25%.

Stable Disease: A <50% decrease to a <50% increase in the sum of the diameter product of all measurable lesions.

Progressive Disease: A ≥ 50% increase in the diameter product of any existing single lesion or the appearance of any new lesions.

## 11.9 APPENDIX 9: SOLID TUMOR RESPONSE CRITERIA (RECIST)

1. The CTEP response criteria for solid tumors (RECIST) outlined below will be used to assess response:

2. Only patients with measurable disease at baseline can have objective tumor response evaluated as an endpoint.

- Measurable disease: The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology, if possible.
- Measurable lesions: Lesions that can be accurately measured in at least one dimension with longest diameter $\geq$ 20 mm using conventional techniques or $\geq$ 10 mm with spiral CT scan.
- Non-measurable lesions: All other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan), bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.

3. All measurable lesions up to a maximum of five lesions per organ and ten lesions in total, representative of all involved organs should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate repeated measurements.

4. All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

5. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

6. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

7. Tumor Response:

- Complete Response: the disappearance of all target lesions
- Partial Response: at least a 30% decrease in the sum of the longest diameter of all target lesions
- Progressive disease: at least a 20% increase in the sum of the longest diameter of all target lesions
- Stable disease: neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease

From: *Therasse, P., et al., New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst, 2000. 92(3): p. 205-16*

## 11.10 Appendix 10: Filgrastim Dosing for Donors Prior to Apheresis

**Minor Donors:**

The donor will receive filgrastim as an outpatient at a dose of 10 μg/kg/day each morning by subcutaneous (SQ) injection beginning 4 days prior to scheduled collection. The donor and/or a family member will be taught to administer the SQ injections when possible. Otherwise, injections will be given in the POB outpatient clinic. The filgrastim should be given as early as possible after awakening in the morning. This is especially important on days 5, 6, and 7 of the injections. Filgrastim will be continued for 5, 6, or 7 days as required to complete collection of the target dose. The donor will continue filgrastim for the complete 7-day period, unless notified by the transplant team that the target dose was reached before day 7.

**Adult Donors (>18 years) :**

Timing of dose: Doses for days 1-4 should be given as early as possible after awakening in the morning, but the dose for day 5 and if necessary, days 6 and 7 must be given early in the morning, at least one hour and preferably two hours prior to starting apheresis.

Filgrastim is administered according to a vial-based algorithm to reduce wastage. Two dosing algorithms are recommended for use in the Clinical Center. The standard dose algorithm uses doses in the range of 10 to 12 mcg/kg /day, with a higher dose given to lighter weight donors to improve CD34 yields. The higher dose algorithm will be utilized in this protocol as it is intended for use in (1) autologous donors who have received prior myelotoxic agents; **(2) donors whose components will undergo further processing; (3) protocols in which a high transplant cell dose is required (CD34 > 8 x$10^6$); and** (4) situations with large weight discrepancy between donor and recipient (recipient > donor). The total dose is capped at 1200 mcg/kg/day.

Higher-Dose Filgrastim Algorithm:

| Donor Wt | Total Daily Filgrastim Dose (range) | |
|---|---|---|
| 38 - 48 kg | 600 mcg | (12.5 to 15.8 mcg/kg) |
| 49 - 56 kg | 780 mcg | (13.9 to 15.9 mcg/kg) |
| 57 - 60 kg | 900 mcg | (15.0 to 15.8 mcg/kg) |
| 61 - 67 kg | 960 mcg | (14.3 to 15.7 mcg/kg) |
| 68 - 108 kg | 1080 mcg | (10.0 to 15.9 mcg/kg) |
| > 109 kg | 1200 mcg | (11.0 or less) |

Predictable side effects of filgrastim, including headache, bone pain, and myalgia, may be treated with acetaminophen or ibuprofen.

## 11.11 APPENDIX 11: ENGRAFTMENT SYNDROME (SPITZER, 2001)

Diagnosis of Engraftment Syndrome (ES) is made based on the presence of all three (#) major criteria, or two (2) major criteria with one or more minor criteria.

*Major Criteria*

- Temperature ≥ 38.3◦C with no identifiable infectious etiology
- Erythrodermatous rash involving more than 25% of body surface area and not attributable to a medication
- Noncardiogenic pulmonary edema, manifested by diffuse pulmonary infiltrates consistent with this diagnosis, and hypoxia.

*Minor Criteria*

- Hepatic dysfunction with either total bilirubin ≥ 2 mg/dl or transaminase levels ≥ two times ULN
- Renal insufficiency (serum creatinine ≥ two time baseline
- Weight gain ≥ 2.5% of baseline body weight
- Transient encephalopathy unexplainable by other causes.

In addition, ES should occur within 96 hours of engraftment (defined as a neutrophil count of ≥ 500/μL for 2 consecutive days).

## 11.12 APPENDIX 12: TBI GUIDELINES

- TBI will be delivered to a total midplane dose of 12Gy and median lung dose of 6Gy. Radiation will be fractionated twice daily over 3 days (days -6, -5, -4). Opposed lateral fields with lung compensation will be treated to 2 Gy for each fraction to a total cumulative dose of 12Gy.

- Mediastinal fields will be treated AP-PA concurrently with the opposed lateral fields at a dose of 100cGy twice daily to bring the mediastinal dose to 12Gy with a median lung dose of 6 Gy.

- Fractions will be delivered with at least a 6 hour interfraction interval. Gonadal shielding will not be used. Head and neck compensation and other technical modifications may be made at the discretion of the treating radiation oncologist.

- For patients with CNS positive disease at any time after diagnosis, CNS boost may be delivered unless contraindicated. The final decision of appropriateness of delivering the boost and boost dose will be made by the PI in consultation with the treating radiation oncologist. The CNS boost will be delivered as a 6 Gy boost in once daily 2 Gy fractions. The boost will be delivered prior to TBI.

- Patients with sites identified by nuclear imaging or other clinically accepted diagnostic tests may receive a boost to these areas as clinically indicated at any time point pre- or post transplantation.

## 11.13 APPENDIX 13: CALCULATION OF IDEAL BODY WEIGHT AND DOSE ADJUSTMENTS

Formulation for deriving the weight to be used in dose calculations in morbidly obese candidates.

1. **Definition**

Obesity is defined as a > 125% of IBW.

2. **Calculation of ideal body weight** is performed using the standard, published formula:

**Male**: 50 + 2.3(Hgt - 60) where Hgt is in inches, and the result is expressed in kg.

ex. The ideal weight of a 5'10" male = 50 + 2.3(10) = 73 kg.

**Female**: 45.5 + 2.3(Hgt - 60), where height is in inches, and the result is in kg.

3. Dose adjustments for pre-transplant myeloablative chemotherapy will be based on an adjusted weight.

**Adjusted weight** =1.25 x IBW

4. **Calculation of the "practical weight."**

Calculate the midway point, halfway between the actual and ideal body weights (ie the average of the two numbers). This is the "practical weight" which may be used for calculating the targeted cell dose.

**Example**:

Patient's actual weight = 143 kg.

Patient' s actual height 173 cm = 69 in

IBW formula = 50 + 2.3(9) = 70.7 kg

Midway point between 70.0 and 143 = 107 kg.

**The weight we would use in targeting cell dose is 107 kg.**