CLINICAL RESEARCH PROJECT
Protocol #99-H-0064

Closed to new accrual and follow up care June 2008

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Title: Exploratory study of non-myeloablative allogeneic stem cell transplantation and donor lymphocyte infusions for metastatic neoplasms refractory to standard therapy.

Other identifying words: Peripheral blood stem cells, non-myeloablative bone marrow transplantation, metastatic solid tumors, engraftment, graft-versus-host disease, graft-versus leukemia, graft-versus-tumor, cyclophosphamide, fludarabine, donor apheresis.

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Subjects of Study:	Number	Sex	Age range
	up to 86	either	Recipients: 10-80 (both inclusive) Donors: 10-80

Project involves ionizing radiation?	No
Off site project?	No
Multi-institutional project?	No
DSMB Involvement?	Yes

PRECIS

The main objective of this study is to identify metastatic neoplasms, which may be susceptible to the graft-versus-tumor (GVT) effect. We will treat patients with progressive metastatic solid tumors refractory to standard therapy with a non-myeloablative allogeneic peripheral blood stem cell (PBSC) transplant from a family donor. A GVT effect from immunocompetent donor immune cells could extend life expectancy and possibly cure such patients.

Eligible subjects will be treated with an allogeneic peripheral blood stem cell transplant from an HLA identical or single HLA antigen-mismatched family donor, using an intensive immunosuppressive regimen without myeloablation ("mini-transplant") in an attempt to decrease the transplant related toxicities while preserving the anti-malignancy and/or anti-host marrow effect of the graft. The low intensity non-myeloablative conditioning regimen should provide adequate immunosuppression to allow stem cell and lymphocyte engraftment. A T -cell replete, donor-derived, granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC) will be used to establish hematopoietic and lymphoid reconstitution. We will infuse lymphocytes in patients with <100% donor T-cell chimerism or with evidence of tumor progression in an attempt to prevent graft rejection and enhance a graft-versus-malignancy effect, respectively.

This trial is open to several different types of metastatic, treatment-refractory, solid neoplasms (breast, cholangiocarcinoma, small intestine/colon/rectal adenocarcinoma, esophageal/gastric, hepatocellular,-pancreatic, prostate, and bony/soft tissue sarcomas). The trial design permits up to 10 subjects with a specific tumor type to be enrolled to screen for anti-tumor effects. A single complete response in a specific tumor type is an indication to exclude further subjects with that diagnosis from the study. Subsequently, a new protocol which focuses on further defining a GVT effect in that disease category will be instituted.

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SEE ALSO **HB PBSCT SUPPORTIVE CARE GUIDELINES (Supportive Care Guidelines)**
Marrow stem cell transplant section
Cellular and Molecular Therapeutics Branch, NHLBI
IRB reviewed 10/21/2003 and 6/6/2006

1.0 OBJECTIVES

1.1 To identify an anti-tumor effect of allogeneic PBSC transplantation by induction of a graft-versus-tumor (GVT) effect in subjects with a diversity of metastatic solid tumors, which are refractory to standard therapy.

1.2 To evaluate engraftment by bone marrow and blood chimerism analysis.

1.3 To evaluate the effects of donor lymphocyte infusion (DLI) and cyclosporine A (CSA) withdrawal on tumor regression.

2.0 BACKGROUND

2.1 Introduction

Despite a number of advances in the treatment of solid tumors, the majority of patients who develop metastatic disease die from their underlying cancer. With the exclusion of testicular cancer, germ cell tumors, and some lymphomas, solid tumors which have progressed to overt metastatic disease remain largely incurable. Although a number of new and novel therapies have been developed to treat patients with advanced metastatic diseases, none to date have been shown to be curative and there is no good evidence that any of these therapies prolong survival.

There exists overwhelming evidence, both in-vitro and in-vivo, that metastatic solid tumors may be susceptible to immune-mediated control (1,2,3). The best support for this immune response comes from the demonstration of an autologous anti-tumor response in melanoma and renal cell carcinoma (RCC) (11). Tumor-infiltrating lymphocytes (TIL) can be found in a majority of metastatic melanoma lesions (27). These TILs can be expanded in-vitro from melanoma tissue and have been shown to consist of a predominant population of cytotoxic lymphocytes (CD8+). Expanded TIL populations have potent MHC-restricted and non-restricted cytolytic activity against autologous tumor while sparing normal host tissue (30). Transfer of cultured TIL along with IL-2 has resulted in tumor regression in 30%-40% of patients with metastatic melanoma (11). A large body of literature exists supporting the in-vitro susceptibility of other metastatic solid tumors to T-lymphocytes. TIL, analogous to those seen in metastatic melanoma, have been isolated from a diversity of other metastatic solid tumors and have been shown in-vitro to be capable of inducing tumor-specific lysis (4,5,6,7,8,9). These findings have led to a number of novel clinical trials aimed at generating autologous tumor-specific T -cells as a form of cancer immunotherapy. Indeed, a number of cancer centers have shifted away from standard chemotherapeutic trials to exploring these new immunotherapeutic approaches utilizing T-cells as the principal effectors.

It is now well established that chronic myelogenous leukemia (CML), myelodysplastic syndromes (MDS), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), Hodgkin's and non-Hodgkin's lymphomas and multiple myeloma can be cured by allogeneic bone marrow transplantation (BMT) (22,23). This curative effect has been ascribed to the use of myeloablative chemo-radiotherapy and the antileukemic effect of the transplant (the graft-versus-leukemia, GVL, effect) (24). The assumption that the intensive myeloablative preparative regimen is essential for the cure of the malignancy went unchallenged until the demonstration by Kolb et al, subsequently confirmed by numerous investigators, that donor lymphocytes alone exert a powerful antileukemic effect in the context of patients relapsing with myeloid leukemias after BMT (20,21,25,26). This observation has had important implications: first, it now appears that some hematological malignancies can be cured with preparative regimens of lower intensity, designed to immunosuppress the recipient to allow lymphocyte and stem cell engraftment without major cytoreduction of the malignancy by myeloablation (17); second,

because low-intensity preparative regimens have decreased toxicity, allotransplantation is now being formed in patients who were previously not considered candidates for standard "myeloablative" procedures due to prohibitive procedure-related mortality (15). Several groups, including our own, have begun to investigate this approach to expand the applicability of allotransplantation to other patients (14,15,17). Our own experience at the NIH includes over 100 patients and has also been favorable, with a low incidence of treatment-related complications and only one treatment-related death in a cohort at high risk for mortality with standard allotransplantation (15). The decreased toxicity of non-myeloablative regimens now makes allogeneic PBSC transplantation appropriate as experimental therapy for patients with metastatic solid tumors refractory to standard therapy who have no other potentially curative treatment option.

There are a number of logical reasons to evaluate allogeneic PBSC transplantation in patients with metastatic solid tumors. The allograft provides the opportunity to attack the tumor with the donor immune system, which is intact and unsuppressed by chemotherapy and has yet to develop tolerance to the malignancy. In addition, patients with growing tumors may lack the T-cell repertoire necessary to recognize and destroy the tumor, a situation analogous to what is sometimes seen in patients with leukemia. Finally, over and above the presentation of known tumor-specific antigens, the malignant cells may present a diversity of allo-antigens to the donor immune system, which have the potential for inducing powerful immunodominant responses.

Recently, we and others explored the possibility of generating anti-tumor effects with allogeneic T-cells following HLA matched peripheral blood stem cell transplantation. Preliminary data indicate that reproducible GVT effects can be generated in patients with RCC, malignant melanoma, and breast carcinoma (1,2,15). In addition, anecdotal reports exist of GVT effects in a number of other tumors including gastric carcinoma, neuroblastoma, and hepatocellular carcinoma.

The melanoma and RCC non-myeloablative allogeneic PBSC transplant trials continue to accrue patients and show clear evidence that GVT effects can be generated in these tumors. There is reason, therefore, to believe that many other tumors may be susceptible to this approach.

The focus of this study is to identify other solid tumors, which may respond to this treatment approach. We now plan to evaluate low-intensity blood stem cell transplants in all patients with incurable metastatic solid tumors refractory to standard therapy. The design of the study incorporates the following features:

1) This is a phase II trial to determine the therapeutic potential of a new transplant approach (GVT effect) on incurable metastatic neoplasms which are refractory to conventional therapy.

2) Patients to be studied:

   The original protocol included 15 groups of neoplasms (adrenal, basal cell, breast, transitional cell carcinoma of the bladder or uroepithelium, cholangiocarcinoma, small intestine/colon/rectal adenocarcinoma, esophageal/gastric, hepatocellular, ovarian, pancreatic, prostate, bony/soft tissue sarcomas, small cell lung cancer, non small cell lung cancer, and adenocarcinomas of unknown primary origin).

   December 2006, the protocol was amended to remove adrenal, basal cell, transitional cell carcinoma of the bladder or uroepithelium, ovarian, small cell lung cancer, non small cell lung cancer and adenocarcinomas of unknown primary origin due to low accrual issues. In the amended protocol patients with the following 8 groups of metastatic solid tumors (breast, cholangiocarcinoma, small intestine/colon/rectal adenocarcinoma, esophageal/gastric, hepatocellular, pancreatic, prostate,

bony/soft tissue sarcomas) refractory to standard therapy, or in which no treatment modality has been shown to be curative or to prolong survival will be eligible for study.

Many of these patients would have received prior dose-intensive chemotherapy and/or radiotherapy and would be at increased risk for regimen-related toxicity and mortality with a standard myeloablative transplant. Because the transplant does not incorporate intensive chemotherapy and relies instead on a GVT and a graft-versus-marrow effects, which may take months to be effective, only patients with expected survivals of 3 months or greater will be eligible for study. Currently there exists no curative option for such patients.

3) Immunosuppression without myeloablation: Patients will receive chemotherapy sufficient to allow donor lympho-hematopoietic engraftment without complete marrow ablation. If the graft is rejected, the patient will reconstitute autologous marrow function to pre-transplantation levels. We will use a combination of two or three agents with known immunosuppressive activity in marrow transplantation: cyclophosphamide, which produces transient pancytopenia; fludarabine, which causes less cytopenia but equivalent immunosuppression; and in patients at increased risk for graft rejection (section 7.5.1) antithymocyte globulin (ATG), which is potently immunosuppressive.

4) Peripheral blood hematopoietic progenitor cell (PBPC) transplant: An unmanipulated peripheral blood stem cell collection from a G-CSF stimulated HLA-matched family donor should improve the chance of engraftment because of the high stem cell doses infused (>5 x 10^6/kg CD34+ cells). In addition, the allograft will be T -cell replete, which should improve donor engraftment and facilitate GVT effects. Patients will receive cyclosporine A (CSA) and methotrexate (MTX) as graft versus host disease (GVHD) prophylaxis post-transplant. Patients with mixed T -cell chimerism at day 30 will be tapered off CSA over 2 weeks, and patients with 100% donor T -cell chimerism will begin a CSA taper at day 60 (through day 100) if no GVHD is present. Patients with evidence of disease progression without grade ≥ II GVHD will have CSA discontinued as early as day 30 regardless of chimerism status.

5) Donor lymphocyte infusion (DLI): Patients who show progressive disease off of CSA and in the absence of grade ≥ II GVHD, or who are at risk for graft failure due to incomplete donor T-cell engraftment will receive one or more DLI as outlined in section 7.11.

3.0 SCIENTIFIC AND CLINICAL JUSTIFICATION

This pilot study will evaluate whether allogeneic PBPC transplants have any useful anti-tumor effects in a diverse group of metastatic solid tumors, which are progressive and refractory to standard therapy. We have in-vitro and in-vivo evidence that GVT effects can be generated using this transplant approach in metastatic breast cancer, melanoma and RCC (1,2,15,27). Many other solid tumors have characteristics that would make them susceptible to this same GVT effect, including class I MHC expression necessary for the presentation of minor histocompatibility antigens and aberrantly expressed mutated peptides (29,30). This experimental approach is ethically justifiable since we are targeting patients with progressive metastatic neoplasms in which no known standard therapy is potentially curative or definitely capable of extending life expectancy. These patients have no prospect of prolonged survival with any standard treatment approach, and are therefore, candidates for this type of investigational therapy.

There is sufficient experimental evidence and clinical data to indicate that the low dose preparative regimen is capable of allowing lympho-hematopoietic engraftment with minimal toxicity. Cyclophosphamide alone permits engraftment in patients with severe aplastic anemia (9), and fludarabine has been used either alone or in conjunction with Atgam and other agents to achieve engraftment (10).

Early data indicate that non-myeloablative stem cell transplants are associated with an excellent transplant outcome in patients with hematological malignancies. The presence of high stem cell doses and donor lymphocytes is known to facilitate engraftment (18,19). Preliminary studies, including our own experience with PBPC transplants and low intensity preparative regimens, indicate that transplant-related mortality (TRM) and severe acute GVHD is uncommon when matched family donors are used.

4.0 STUDY DESIGN

The subject will receive a preparative regimen of cyclophosphamide 60 mg/kg/d IV x 2 days, followed by fludarabine 25 mg/m^2/d iv over 30 minutes x 5 days (+/- Atgam 40 mg/kg IV x 4 days -5 to -2: section 7.5.1-7.5.2), followed by a PBPC graft targeted to deliver >5 x 10^6 CD34+ cells /kg. CSA and MTX for GVHD prophylaxis will be used in all subjects. An HLA-matched family donor will receive G-CSF SC for 6 days with apheresis collections of PBPC on days 5 and 6 (and day 7 if required).

CSA beginning day -4 and intravenous MTX on days +1, +3, and +6 will be given. Subjects with mixed T-cell chimerism on day 30 will begin a CSA taper. Subjects with 100% donor T-cell chimerism by day 30 will be tapered off CSA from days 60 through 100 (25% reduction in dose every 10 days-off by day 100). CSA will not be tapered in any subjects with grade ≥ II acute GVHD regardless of chimerism results. In addition, subjects with evidence of disease progression without grade ≥ II GVHD will have CSA discontinued regardless of chimerism results.

On days, +30, +60 and +100, disease status will be assessed by physical exam and by computerized tomography (CT) scans, and subjects with disease progression without grade ≥ II GVHD will have CSA discontinued and may receive DLI as outlined in section 7.11.

In addition, on days +30, +60 and +100, the chimeric status of subjects will be assessed by minisatellite analysis of the peripheral blood and sometimes bone marrow. Subjects demonstrating less than 100% donor T-cell chimerism on day 30 will be tapered off CSA over a 12 day period and will be re-evaluated. If T-cell chimerism remains less than 100% donor, infusions of previously collected and cryopreserved donor lymphocytes will be given in an attempt to prevent graft rejection in the absence of grade ≥ II GVHD.

5.0 ELIGIBILITY ASSESSMENT

5.1 Inclusion criteria -patients

5.1.1 Patients with metastatic solid tumors breast, cholangiocarcinoma, small intestine/colon/rectal adenocarcinoma, esophageal/gastric, hepatocellular, pancreatic, prostate, bony/soft tissue sarcomas) which are histologically confirmed, progressive and incurable.

Due to low accrual, effective 12/19/2006, patients with adrenal, basal cell, transitional cell carcinoma of the bladder or uroepithelium, ovarian, small cell lung cancer, non small cell lung cancer, and adenocarcinomas of unknown primary origin are no longer eligible for the trial (see also section 13.1).

5.1.2 Age ≥ 10 to ≤ 80.

5.1.3 No known standard therapy for the patient's disease that is potentially curative or definitely capable of extending life expectancy.

5.1.4 Metastatic disease, which is bi-dimensionally evaluable radiographically.

5.1.5 No prior treatment for neoplasm within 30 days.

5.1.6 Ability to comprehend the investigational nature of the study and provide informed consent.

5.1.7 Availability of HLA identical or single HLA-locus mismatched family donor.

5.1.8 Willingness and availability to return to the NIH for scheduled follow ups.

5.2 Exclusion criteria for the patient: any of the following

5.2.1 Pregnant or lactating.

5.2.2 Age <10 or >80 years.

5.2.3 ECOG performance status of 3 or more, (see Supportive Care Guidelines).

5.2.4 Psychiatric disorder or mental deficiency severe as to make compliance with the BMT treatment unlikely, and making informed consent impossible.

5.2.5 Major anticipated illness or organ failure incompatible with survival from PBSC transplant.

5.2.6 DLCO: < 40% predicted.

5.2.7 Left ventricular ejection fraction: < 30%.

5.2.8 Serum creatinine > 2.5mg/dl or creatinine clearance < 50 cc/min by 24 hr urine collection.

5.2.9 Serum bilirubin >4 mg/dl.

5.2.10 Transaminases > 5x upper limit of normal.

5.2.11 Oral intake < 1,200 calories/day.

5.2.12 Recent weight loss of ≥10% of actual body weight.

5.2.13 Life expectancy < 3 months.

5.2.14 Therapy for malignancy within 4 weeks of beginning protocol.

5.2.15 CNS metastatic disease associated with intracranial bleeding, uncontrolled seizure disorder or significant intracranial mass effect.

5.2.16 Other malignant diseases liable to relapse or progress within 5 years.

5.2.17 Uncontrolled infection.

5.3 Inclusion criteria - Donor

5.3.1 HLA identical or single HLA-locus mismatched family donor

5.3.2 Age ≥ 10 up to 80 years old

5.3.3 Ability to comprehend the investigational nature of the study and provide informed consent.

5.4 Exclusion criteria for the donor: any of the following

5.4.1 Pregnant or lactating

5.4.2 Donor unfit to receive G-CSF and undergo apheresis. (Uncontrolled hypertension, history of congestive heart failure or unstable angina, thrombocytopenia)

5.4.3 Age <10 or >80 years

5.4.4 HIV positive. Donors who are positive for HBV, HCV or HTLV-I may be used at the discretion of the investigator following counseling and approval from the recipient.

6.0 CLINICAL EVALUATION OF THE SUBJECT

Human Biologic materials collected as follows are for the clinical evaluation and management of the subject. The biopsy of the primary neoplasm and metastatic disease, and the bone marrow aspiration and biopsy will be read by a pathologist. Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

6.1 Pre-Study Evaluation

6.1.1 Complete history and physical exam.

6.1.2 Biopsy confirmation of primary neoplasm and metastatic disease. Histology slides will be kept on file in the pathology department of the clinical center while the subject is being treated on protocol.

6.1.3 CT scans of the chest, abdomen and pelvis, MRI scan of the brain, as well as any other appropriate radiographic or nuclear medicine studies to document evaluable disease within 30 days of induction of the preparative regimen. Subjects with tumors involving the extremities including soft tissue and bony sarcomas will have baseline MRI and/or CT studies of involved regions.

6.1.4 Medium resolution molecular HLA- A, B, DR typing.

6.1.5 Bone marrow aspiration and biopsy with chromosome analysis, PCR, and flow cytometry as appropriate to stage and classify underlying disorder.

6.1.6 Antibody screen for HBV, HCV, HIV, HTLV-I/II, CMV, EBV, toxoplasma, syphilis. PPD test for subjects from areas where tuberculosis is prevalent.

6.1.7 Coagulation screen, CBC with differential.

6.1.8 Acute care panel, hepatic panel, and mineral panel.

6.1.9. Chest radiograph, pulmonary function testing: vital capacity FEV-1, DLCO.

6.1.10 Sinus CT scan.

6.1.11 Cardiac function: EKG, MUGA scan, baseline 2-D echocardiogram.

6.1.12 All subjects age 50 or greater, or age 40 or over, with one of the following risk factors: a history of high blood pressure, increased cholesterol, smoking, or diabetes or family history of coronary disease, will have a baseline cardiac workup, which will include:

1. Stress nuclear perfusion imagery.
2. Cardiac consultation in subjects age 50 and over.

6.1.13 Nutritional assessment.

6.1.14 Dental review.

6.1.15 Social worker interview.

6.1.16 Ophthalmology consultation.

6.1.17 Interview with members of primary care team and visit to unit.

6.1.18 Consent form signed.

6.1.19 Durable power of attorney form completed.

6.1.20 For subjects with accessible tumors, biopsies will be performed to generate tumor lines for in-vitro analysis of donor derived GVT effects

6.2 In Patient Monitoring

Once daily: CBC with differential, acute care, hepatic, and mineral panels, direct bilirubin, temperature, pulse, blood pressure, respiratory rate, weight, caloric intake, abdominal girth.

Twice weekly: reticulocyte, pre-albumin, coagulation screen.

Weekly: CMV surveillance; C-reactive protein; Stool for *C difficile* when appropriate; Drug levels when appropriate (e.g., gentamicin, vancomycin, cyclosporine).

6.3 Follow Up to day 100: Out-patient

At least weekly: CBC, acute care panel, hepatic and mineral screen, LDH, coagulation screen, Temperature, pulse, blood pressure, respiratory rate, weight, C-reactive protein, CMV surveillance. A complete physical exam will be repeated weekly.

Peripheral blood will be drawn weekly until day +60 and in some subjects bone marrow aspirates will be taken on days +15, +30, +45, +60 and +100 to assess for donor-host chimerism in the lymphoid and myeloid cell lines. A sample of peripheral blood will also be obtained for chimerism analysis at the time of neutrophil recovery (ANC>500).

Radiographic follow up: CT scans and other radiographic studies of areas of evaluable disease will be repeated on days +30, +60, +100. CT scans will then be performed every 3 months
Clinic visits: Day +30 to day +100 up to 2x/week or at subject's request.

6.4 Follow Up beyond day 100

At 6, 12, 18, 24, 36, 48 and 60 months (+/- 1 month): CBC, acute care panel, hepatic and mineral screen, chest radiograph, bone marrow aspirate (if applicable) and blood sample for chimerism studies and karyotype, pulmonary function tests.

Radiographic follow up: CT scans and other radiographic studies of areas of evaluable disease will be repeated every 2 months until 1 year post-transplant. CT scans will then be performed every 3 months until 3 years post-transplant; then every 6 months until 5 years post transplant.

Clinic visits: months 4, 6, 8, 10, 12 then every 3 months thereafter until 3 years then every 6 months until 5 years. After 5 years follow up visits are not mandatory but yearly communication with the subject and the referring physician is continued.

7.0 TREATMENT PLAN

7.1 Pre transplant treatment

Subjects with active disease may receive appropriate chemotherapy or radiotherapy according to standard indications to control disease prior to the preparative regimen.

7.2 Apheresis of subject

One collection of 10^{10} leukocytes by a 10-liter leukapheresis before transplant preparative regimen begins and at the time of disease response or acute GVHD for cryopreservation of lymphocytes to be used for laboratory investigation of the graft-versus-malignancy or graft-versus-marrow effect or GVHD effectors.

7.3 Central venous line placement.

A triple lumen Hickman catheter will be placed by an interventional radiologist or a surgeon.

7.4 Infection Prophylaxis and Fever Regimen (see Supportive Care Guidelines)

7.5 Preparative regimen (Appendix A)

7.5.1 Antithymocyte Globulin (ATgam)

Subjects will receive one of two preparative regimens as described below. Subjects at higher risk for graft rejection with this non-myeloablative approach (including heavily transfused subjects or subjects who have received donor directed blood products, and single HLA locus mismatched subjects) will have Atgam added to the preparative regimen.

7.5.2 Dose Levels

1) Cyclophosphamide 60 mg/kg/d IV day -7, -6
Fludarabine 25 mg/m^2/d IV day -5, -4, -3, -2, -1

2) Subjects at increased risk for graft rejection-defined above, 7.5.1
Cyclophosphamide 60 mg/kg/d IV day -7, -6
Fludarabine 25 mg/m2/d IV day -5, -4, -3, -2, -1
Antithymocyte globulin 40mg/kg IV days -5,-4,-3,-2
(Methylprednisolone 1 mg/kg day -5 to + 4 then rapid taper)

7.6 Peripheral blood progenitor cell transplant.

The target for progenitor cells is $\geq 5 \times 10^6$ CD 34/kg.
Minimum dose for transplant is 2×10^6/kg

7.7 GVHD prophylaxis

All subjects will receive CSA and MTX as GVHD prophylaxis. CSA (1.5 mg/kg/dose IV BID) will begin on day -4. Dosing will be based on actual body weight unless the subject is "obese" (BMI > 35) when practical weight will be used. Switch to the equivalent oral CsA dose divided bid on day 14 or when able to tolerate PO. MTX will be given intravenously on days +1, +3 and +6.

Subjects with <100% donor T-cell chimerism on day 30 will begin a 12 day CSA taper (i.e. 25% reduction in dose every 3 days). Subjects with 100% donor T-cell chimerism by day 30 will be tapered off CSA from days 60 through 100 (25% reduction in dose every 10 days-off by day 100). CSA will not be tapered in any subjects with grade ≥ II acute GVHD regardless of chimerism results. In addition, subjects with evidence of disease progression without grade ≥ II acute GVHD will have CSA tapered regardless of chimerism results.

7.8 Transfusion Support (see Supportive Care Guidelines)

Filtered and irradiated blood products. CMV negative recipients of CMV negative or positive marrow will receive CMV negative blood products.

7.9 Nutrition

Parenteral nutrition will be instituted if daily caloric intake <1000 KCal/day in adults ≥ 18 yrs. Subjects 10 to 18 years will start parenteral nutrition if they are unable to meet at least half their daily caloric needs by mouth (i.e. 50 KCal/kg/day).

7.10 Hospital Discharge

Subject will be in the hospital for about 4 weeks and will be discharged when the following criteria are fulfilled:

- Subject afebrile, positive weight balance; no parenteral feeding required.
- Platelet transfusion requirement absent or less than 2x weekly.
- Subject or family able to care for Hickman line.

7.11 Donor lymphocyte Infusions (DLI)

Subjects with disease progression or donor T-cell chimerism of <100% following CSA tapering without grade ≥ II GVHD will receive donor lymphocyte infusions (previously collected and cyopreserved) based on the following algorithm:

Mixed chimerism without disease progression or active GVHD

Monthly increments of DLI 5 x 10^6, 1 x 10^7, 5 x 10^7 /CD3/kg.

Disease Progression (independent of chimerism status) without active GVHD

DLI 1 x 10^7 and repeat doses depending on response and acute GVHD.

8.0 DONOR EVALUATION AND PLAN

Human Biologic materials collected as follows are for the clinical evaluation and management of the donor. Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

8.1 Pre-study consult and evaluation

- HLA-A,-B,-DR typing.
- Confirm HLA identity of donor with recipient (subject).
- History and physical examination.
- Hepatitis B, C, HIV, HTLV-I/II, CMV antibodies, RPR.
- CBC, coagulation screen, acute care, hepatic, mineral screen.
- Fit to donate: Orientation -visit to Department of Transfusion Medicine, Inspection of veins to determine the need for a central line for apheresis.

8.2 Pre-consent evaluation and concurrent care of minor donors (donors less than age 18 only)

For donors less than age 18, a social worker and mental health specialist (psychologist or psychiatrist) will meet with the minor donor prior to the assent process to determine willingness to participate.

Donors who are less than 18 years of age will see a pediatric provider (pediatrician, pediatric nurse practitioner or pediatric physician's assistant) who is separate from the transplant team. This practitioner will serve as the donor's health care provider and advocate during the minor's participation on the clinical trial.

8.3 Donor lymphocyte collection

Prior to undergoing G-CSF mobilization, the donor may undergo a ten liter apheresis for cryopreservation of donor lymphocytes to be infused as necessary after transplantation (section 7.11). Aliquots of 5 x 10^6 CD3/kg, 1 x 10^7 CD3/kg and 5 x10^7 CD3/kg will be cryopreserved. Any remaining cells in excess of that required for cryopreservation will be either discarded or used for GVT studies in vitro (section 11.1).

8.4 Donor mobilization with G-CSF

After medical evaluation and clearance for suitability as an allogeneic donor by the BMT service in consultation with DTM, the donor will undergo mobilization with filgrastim (G-CSF) as an outpatient. Filgrastim (G-CSF) will be administered based on body weight (see below) for 5-6 days, subcutaneously. G-CSF will be administered according to a vial based algorithm to reduce wastage, improve donor compliance, and optimize CD34 yields. The doses for days 1-4 may be given at any time of day, but the doses for days 5 and 6 must be given very early in the morning, prior to apheresis.

Donor Wt Total filgrastim (G-CSF) 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)

The target for progenitor cells is $\geq 5 \times 10^6$ CD 34/kg.
The target CD34+ cell dose is 3×10^6/kg
Minimum dose for transplant is 2×10^6/kg

8.5 Peripheral blood stem cell collection & processing

Leukapheresis procedures will be carried out in the Apheresis Unit, Department of Transfusion Medicine (DTM). These procedures will be done via a 2-armed approach or by temporary central venous catheter in the femoral position, using the Baxter CS3000Plus instrument or the Cobe Spectra. 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 G-CSF and the CD34 cell dose needed, based on kilogram weight of recipient. This will range from 15 to 35 liters processed per day for 2 to 3 days, not to exceed a total of 75 liters over 3 days. In pediatric subjects, defined as less than 40 kg, a maximum of 8 blood volumes will be processed per day, for up to 2-3 days. The second day apheresis collection will not be required if the CD34 dose is 3 $x10^6$/kg or more after the first day of collection.

Anticoagulation will be accomplished with ACD-A, routinely used in normal donors. If the donor is small or intolerant to ACD-A, and the adverse citrate effects cannot be controlled by usual means (slowing flow rate, oral or IV calcium), consideration will be given to using heparin anticoagulation. Donors will receive divalent cation prophylaxis to prevent citrate toxicity during apheresis, in accordance with standard DTM policies.

If the minimum CD34+ cell dose (2×10^6 CD34/kg) is not achieved with one mobilization and 2 apheresis collections, then the PI will consider options of a bone marrow collection, a second course of mobilization and apheresis, or no further donor collections and taking the subject off protocol.

The PBSC collections will not be manipulated except for plasma removal or red blood cell depletion as needed for ABO or other red blood cell antigen incompatibility. ABO or other erythrocyte incompatibility is not an issue because the resulting number of contaminating erythrocytes is well below 1 ml using this system. The final cell suspension will be prepared for infusion by transferring the cells into a 50-150 ml volume of an infusible solution (Plasmalyte A with 1% human serum albumin), suitable for intravenous infusion over 15 minutes to 1 hour, depending on clinical circumstances. PBSC will be either cryopreserved for later thawing and infusion, or infused as fresh products within 48 hours of collection.

On rare occasions, subjects may develop marrow failure as a consequence of graft-vs-host hematopoiesis or from post transplant drug treatment (i.e. ganciclovir). When such situations arise, subjects may require a stem cell "boost". Donors will undergo a repeat mobilization with G-CSF (as outlined above) followed by CD34+ selection of hematopoietic progenitor cells prior to being infused.

8.5 Bone marrow harvest

An occasional donor may not mobilize PBPC well. If the subject has already received the conditioning regimen, and if analysis of the stem cell content of the first or second apheresis products makes it unlikely

that additional apheresis will achieve the minimal CD34 dose, the donor will undergo a bone marrow harvest under general anesthesia the day following the last apheresis.

9.0 MANAGEMENT OF SUBJECT COMPLICATIONS

The major complications, are cytomegalovirus (CMV) reactivation, acute and chronic GVHD, progression of the original disease, donor-recipient ABO incompatibility and pulmonary engraftment syndrome. Subjects with these complications will be treated along the following lines:

9.1 CMV reactivation (See Supportive Care Guidelines)

9.2 Acute GVHD (See Supportive Care Guidelines)

9.3 Chronic GVHD (See also Supportive Care Guidelines)

Cyclosporine at standard dose (See Pharmaceuticals, section 16.3).

Prednisone 20-60 mg daily according to severity.

Change to alternate day steroid and cyclosporine therapy when response is established.

Non-responding subjects may be treated with other standard of care therapies such as azathioprine, sirolimus, tacrolimus, rituximab, mycophenolate, daclizumab or infliximab PUVA, photopheresis, and/or thalidomide at the discretion of the attending physician.

9.4 Graft rejection

This transplant protocol uses a non-myeloablative preparative regimen. Therefore, auto-recovery is anticipated in subjects who fail to engraft. Subjects who fail to demonstrate donor T-cell engraftment will be taken off study and referred back to their primary physician for further therapy.

9.5 Progression of metastatic disease

See section 7.7 and 7.11. Subjects who develop progressive disease despite CSA withdrawal and DLI, and who remain without grade ≥ II acute GVHD may be treated at the discretion of the principal investigator (PI) with standard of care treatment options (i.e. interferon-alpha, low dose IL-2 by subcutaneous injection, GM-CSF, and/or chemotherapy) with or without stem cell rescue.

Subjects with disease progression and grade ≥ II acute GVHD will be taken off study and referred back to their referring physician for care.

9.6 Donor-recipient ABO incompatibility (see PBSCT Supportive Care Guidelines)

9.7 Pulmonary Engraftment Syndrome: Subjects who develop pulmonary engraftment syndrome (most likely 10-14 days post-transplant) will be treated with steroids.

10.0 STUDY MODIFICATIONS

Graft failures will be monitored throughout the study. Specific need for preparative regimen modifications based on the incidence of graft failures will be made as discussed in section 7.5

11.0 ANCILLARY LABORATORY RESEARCH STUDIES

11.1 Sample collection

During the course of participating on this study, blood and tissue as detailed below will be collected for correlative laboratory research studies

- *from subject before transplant:* One apheresis collection of approximately 10^{10} leukocytes in a volume of 200 ml.
- *from subject in hospital*: Weekly lymphocyte subsets
- *from subject at planned outpatient visits*: cytotoxic T-cell precursor frequency (CTLPf) assays and lymphocyte subsets up to day +60, then every 2 weeks until day +100 and then at each scheduled follow-up.
- *from donor at time of leukapheresis:* Donor leukocytes obtained from leukapheresis will be used for research studies investigating graft-vs-malignancy effects. The subject's tumor cells and normal lymphocytes will serve as targets for all subsequent testing of donor-anti host responses.
- *Bone Marrow Samples:* An occasional subject will undergo a pre-transplant bone marrow aspirate for disease staging purposes (i.e. extensive small cell lung cancer) or for tumor analysis when there is a high likelihood of marrow space involvement with metastatic disease. These samples will be read by a pathologist. An extra volume (up to 25 ml) of bone marrow aspirate will be collected for research studies at the pre-transplant evaluation and for monitoring minimal residual disease. The cells will be used to investigate lymphocyte interactions with bone marrow progenitor cell proliferation. No marrow aspirates solely for research purposes are planned

11.2 Intended Use

These specimens will not be read by a pathologist or used for diagnostic purposes. Studies will not be used in assessing the primary endpoint but are undertaken for descriptive or exploratory ancillary research as detailed below:

11.2.1 GVT AND GVHD Studies

Blood and serum from subjects and donors will be obtained by leukapheresis to study genomic and proteomic pathways involved in mediating GVHD and in vitro donor T-cell responses to the recipient's tumor. The cytotoxic T-cell precursor frequency (CTlPf) assay will be used to evaluate the donor immune function at various periods after BMT.

A fine needle aspirate of accessible tumors will be performed pre-transplant to establish tumor cell lines for GVT investigations. Regressing metastatic lesions will be biopsied if accessible and analyzed for the presence of alloreacting TILs. The origin of these cells (donor, host, or mixed chimeric) will be evaluated by minisatellite probes.

11.2.2 Chimerism studies

PCR of microsatellites of post-transplant peripheral blood T-lymphocytes, B-cells, NK cells and blood myeloid cells will be assessed in serial fashion to determine the chimeric status of the subject as a function of time. These studies will be repeated after lymphocyte infusions to assess their effect on host chimeric status as well. The relationship between degrees of donor host chimerism, GVHD and tumor response will be analyzed.

11.2.3 Other research labs as described on the List of Laboratory Studies Using Human Subject and Normal Volunteer Samples in the CMTB Approved by the IRB (Appendix D)

We will share the following de-identified samples with Dr. Li Zhang, Senior Scientist, Toronto General Research Institute (TGRI) at University Health Network: peripheral blood stem cells (PBSC), peripheral blood mononuclear cells (PBC,) coded as TRA-PBMC, GRE-PBMC and WYN-PBMC, and coded Epstein-Bar-Virus (EBV) transformed lymphoblastoid cell line (LCL), coded as TRA-EBV-LCL, GRE-EBV-LCL and WYN-EBV-LCL, derived from healthy donors enrolled in protocols 97-H-0196 and 99-H-0064 and 99-H-0050.

11.3 Tracking, storage and disposition of Samples

Storage: Research samples will be stored coded in the secure laboratory of the PI.

Tracking: Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside NIH without IRB notification and an executed MTA.

End of study procedures: Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

Loss or destruction of samples: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

12.0 RESPONSE CRITERIA

12.1 Tumor response criteria

12.1.1 Complete response (CR): disappearance of all signs and symptoms of metastatic disease for a period of at least one month.

12.1.2 Partial response (PR): a 50% or greater decrease in the sum of the products of the longest perpendicular diameters of all measured lesions lasting for a period of at least one month. No new metastatic lesions may appear.

12.1.3 Stable disease (SD): tumor measurements not meeting the criteria of CR, PR, or PD.

12.1.4 Progressive disease (PD): increase of 25% or greater in the sum of the products of the longest perpendicular diameters of all measured lesions compared to the smallest previous measurements, or the development of any new metastatic disease.

13.0 BIOSTATISTICAL CONSIDERATIONS

13.1 Design and sample size

This trial is designed to estimate a novel treatment regimen on inducing disease regression and or remission in subjects with a number of different metastatic solid tumors, all of which are refractory to standard therapy. It is designed to screen neoplasms of specific origin and histologic subtype which may be sensitive to this approach. We plan to enroll a maximum of 10 subjects with a particular cancer. If any subject achieves a complete response (defined Section 12.0) then we will stop accruing subjects in that disease category and plan to write a separate phase II protocol which will evaluate this regimen specifically in subjects with this type of cancer. If we enroll 10 subjects in a specific disease category and see no responses, we will be 94% confident that the proportion of complete responses in that category is no more than 25% and accrual of these subjects will be terminated (32).

In November 2002, a retrospective analysis for all subjects on HB protocols undergoing non-myeloablative allogeneic stem cell transplantation following cyclophosphamide/fludarabine based conditioning was conducted to determine the impact of adding mycophenolic acid mofetil (MMF) to CSA as GVHD prophylaxis. In this analysis we evaluated the incidence and severity of acute GVHD in our first 66 consecutive subjects who received CSA alone (**Group I**) versus the next 78 consecutive subjects who received CSA + MMF (**Group II**). We saw no beneficial impact of adding MMF to CSA on the incidence of acute grade II-IV, acute grade III-IV GVHD or chronic GVHD. As a consequence, we amended the protocol to replace MMF with the more conventional GVHD prophylactic agent MTX as follows:

1) Discontinued the use of MMF as it has clearly failed to reduce the incidence of acute or chronic GVHD.

2) Continued CSA as GVHD prophylaxis using the same dose and scheduling regimen that was used for subjects in Group I and Group II.

3)Added MTX to CSA as GVHD prophylaxis in the same fashion and doses as are currently given on protocol 97-H-0196. CSA and MTX have been used together for over a decade and are considered "standard agents" for GVHD prophylaxis

In December 2006, in response to low accrual and following recommendation of the DSMB and approval of the IRB the types of solid tumors to be included was reduced from 15 (adrenal, basal cell, breast, transitional cell carcinoma of the bladder or uroepithelium, cholangiocarcinoma, small intestine/colon/rectal adenocarcinoma, esophageal/gastric, hepatocellular, ovarian, pancreatic, prostate, bony/soft tissue sarcomas, small cell lung cancer, non small cell lung cancer, and adenocarcinomas of

unknown primary origin) to 8 (breast, cholangiocarcinoma, small intestine/colon/rectal adenocarcinoma, esophageal/gastric, hepatocellular, pancreatic, prostate, bony/soft tissue sarcomas). Therefore, the revised sample size will be a maximum of 86 subjects (8 disease groups x 10 subjects/group) + 6 subjects in no longer accruing disease categories (1 adenocarcinoma, 1 adrenal, 1 basal cell, 1 lung cancer and 2 ovarian cancer subjects).

13.2 Parameters to be monitored

1) Regression of metastatic disease.

2) Degrees of donor-recipient lymphoid and myeloid chimerism by minisatellite probe analysis of peripheral blood and marrow cells (marrow will be collected on days +15, +30, +45, +60, & +100).

3) Neutrophil recovery (days to neutrophil count of 0.5 x 10^9/1 and 1.0 x 10^9/1).

4) Platelet recovery (days to platelet count of 50 x 10^9, days to transfusion independence).

5) Red cell recovery (days to transfusion independence).

6) Effect of CSA and MMF withdrawal and DLI on donor-host lymphoid and myeloid chimerism measured by mini satellite DNA analysis of T-lymphocytes and myeloid cells.

7) Incidence and severity of acute and chronic GVHD.

8) Non-hematological effects attributable to the preparative regimen.

9) Allograft cell dose (CD34+ cell dose, CD3+ cell dose) infused.

13.3 Other endpoints

13.3.1 The proportion of graft failures: The proportion of graft failures will continue to be monitored. If there are 4 graft failures among the first 14 subjects, or 5 among the first 28 subjects, or 6 among the first 42 subjects, we will reassess our preparative regimen because such results are inconsistent with prior beliefs that the graft failure rate is most likely to be 5% and unlikely to be greater than 15%. A beta distribution with parameters 2 and 38 was used as the prior distribution for this calculation.

13.3.2 The proportion of subjects with Grade 3 or higher acute (day 100 or less) and chronic GVHD will also be estimated with 95% confidence intervals.

13.4 Stopping rule for safety

We will monitor 200 day transplant related mortality (TRM) in all of the neoplasms combined and wish to stop this trial if the 200 day TRM proportion is higher than 30-40%. We take a beta distribution with parameters 4 and 6 and for our prior distribution, consistent with the belief that the proportion of TRM at 200 days is centered at .4 and is not likely to be greater than .6 (prior probability .05) or less than .2 (prior probability .03). We stop if the probability that the 200 day TRM is greater than .4 is at least .9. The stopping rule below was assessed by simulation based on the first 80 subjects and results in .7 overall probability of stopping early if the true 200 day TRM proportion is .5, .2 overall probability of

stopping early if the true 200 day TRM proportion is .4, and .02 overall probability of stopping early if the true 200 day TRM proportion is .3. The stopping rule is:

No. subjects	No. TRM
7-10	7
11-20	12
21-30	17
31-40	21
41-50	25
51-60	30
61-70	34

It should be noted that stopping is permitted prior to accrual of a complete cohort of 10 subjects.

13.5 Withdrawal from study

13.5.1 Withdrawal by the subject from the transplant procedure

Subjects and their donors will be given ample opportunity to withdrawal from the study prior to admission for transplant. Thereafter, the nature of the procedure does not permit safe withdrawal from the protocol.

The subject and donor have the right at any time to elect not to participate in the research aspects of the protocol (donation of blood and bone marrow for non-routine tests).

13.5.2 Withdrawal by the physician from experimental protocol

Subjects who fail to achieve allogeneic marrow engraftment (donor chimerism or mixed chimerism) will be removed from study after full autologous marrow recovery is achieved.

Subjects with disease progression after the day +160 restaging who have active grade ≥II GVHD or who have failed to respond to a previous lymphocyte transfusion and/or to interferon-alpha or IL-2 may be removed from study.

Subjects with disease progression associated with a significant decline in performance status which negates further treatment on protocol (i.e. DLI) will be removed from study.

13.6 Data management

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 PI, associate investigators (AI), CMTB fellows, research nurses and/or a contracted data manager will assist with the data management efforts.

All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded in DIR's Clinical Data System (CDS) or the Laboratory of Cardiac Energetics (LCE) database. Primary 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, e.g., study-specific identifying number (SSPIN) generated by CDS or other unique code or minimum PII required for subject identification.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

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

Data will not be sent outside NIH without IRB notification and an executed MTA or CTA. An MTA has been executed and a copy is on file with the Office of Clinical Affairs for the transfer of sample to Pierre Ronco, MD, PhD, Head, Nephrology Department, Unit 702 At Tenon Hospital, Paris, France tel : + 33 1 56 01 66 39/38 pierre.ronco@tnn.aphp.fr.

Publication Policy: Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH Intramural IRB review and approval or an exemption from the NIH Office of Human Subjects Research (OHSR).

14.0 DATA SAFETY AND MONITORING

14.1 Safety Monitoring

*Principal Investigator**:*** Accrual, efficacy, and safety will be monitored by the PI. The study will be continuously evaluated for any unusual or unpredicted complications with the aim of detecting and preventing unacceptable increase in morbidity and mortality over that anticipated from unmanipulated bone marrow transplantation.

NIH Intramural IRB. Accrual and safety data will be reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed patient consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to 45 CFR 46 Code of Federal regulations. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

DSMB: The NHLBI Data Safety and Monitoring Board will review the protocol at six or twelve month intervals. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy. Since this study was closed to accrual and follow up care in June 2008, a final report was submitted to the DSMB and this study is no longer reviewed by the DSMB.

14.2 Adverse Events

The definitions of the Reportable Events will be followed per Policy 801.

NIH IRB and CD reporting
Expedited Reporting
Events requiring expedited reporting will be submitted to the IRB per Policy 801 "Reporting Research Events".
Reports to the IRB at the time of Continuing Review:
The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.
Reports to the CD:
The PI or designee will refer to NHLBI DIR Policy to determine CD reporting requirements and timelines.

DSMB: All SAEs will be included for review by the DSMB at six to twelve month intervals. Reports of serious adverse events that are unexpected and thought to be related to the experimental will also be forwarded immediately to the Data and Safety Monitoring Board (DSMB). All SAEs will be included for review semiannually by the DSMB.

15.0 HUMAN SUBJECT PROTECTION

15.1 Rationale for subject selection

No subjects will be excluded from participation based on gender, race or ethnicity. All patients with eligible diseases itemized in section 5.1 will be considered for the protocol. Because the transplant does not incorporate intensive chemotherapy and relies instead on a GVT and a graft-versus-marrow effects, which may take months to be effective, only patients with expected survivals of 3 months or greater will be eligible for study.

Strategies for patient recruitment: Oncologists throughout the country will be informed of the protocol by letter. Information about the protocol will be posted on Clinicaltrials.gov, Clinical Center Studies, and the NHLBI patient recruitment websites. The protocol will also be listed in the PDQ directory. A recruitment flyer for the protocol will also be submitted to several oncology journals.

15.2 Participation of children

Participation of children as stem cell transplant recipients: In principle, age is not a consideration. In practice, we are limiting the protocol to patients over the age of 10 years as the diseases under study on this protocol (metastatic, treatment-refractory, solid neoplasms (breast, cholangiocarcinoma, small

intestine/colon/rectum, esophageal/gastric, hepatocellular, pancreatic, prostate, bony/soft tissue sarcomas) would be relatively rare in children as compared to adults and an extraordinary effort would be needed to accrue sufficient number of children to access safety and/or efficacy.

*Participation as stem cell donors: :*As the stem cell transplant on this protocol is not an accepted standard clinical intervention for the disease under investigation and the donor would not be donating the stem cells outside the context of this research, the donors become subjects because the investigators are intervening with them through the stem cell harvest procedure. The stem cell collection procedure is considered part of the research for the donors and the risks and benefits of the harvest procedure are considered as risks and benefits of the research for the donors.

As participants in research studies: Pediatric donors may participate only in those laboratory studies that the IRB finds involves no greater than minimal risk to children provided that adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians (see section 15.4.5)

15.3 Hazards and Discomforts

15.3.1 The transplant recipient

Related to the transplant: The mortality from conventional BMT may be as high as 40%. Although our data as well as that of others suggest a significant reduction in transplant related mortality with non-myeloablative PBSC transplantation, the procedure nevertheless carries risk. It is therefore only appropriate to carry out this experimental procedure in the context of life-threatening metastatic cancer, which is incurable with conventional therapy and with full informed consent from the patient and donor. The specific hazards of this study using a non-myeloablative preparative regimen and high PBPC content graft are graft rejection, graft-versus-host disease and progression of metastatic disease. The major discomforts are those of nausea, mucositis, anorexia, diarrhea, fever and malaise, and intolerance of the isolation period.

Related to the common drugs used in BMT:

Antithymocyte Globulin: Febrile reactions, allergic symptoms including rash, wheezing, anaphylaxis, cytopenias, serum sickness, nausea, vomiting, diarrhea, arthralgias, hypotension, tachycardia, bradycardia, fever, chills, myelosuppression. Several cases of a severe lung injury related to ATgam treatment have been reported. Although this side effect appears extremely rare, it is serious and can be fatal. There is no information about the mechanism or specific treatment for this condition. A few patients recovered after intensive medical support including use of a breathing machine.

Cyclosporine: CSA is metabolized primarily in liver but the major toxicity is renal. Side effects include renal impairment, reversible renal insufficiency, hemolytic uremic syndrome, elevated bilirubin and transaminases that normalize with continued administration or reduced dose, hypertrichosis, headaches, nausea, gingival hypertrophy, parasthesias (painful hands and feet), hypertension, hypomagnesium, bilirubinemia, hypertrichosis, nausea, tremor, and seizure. An extremely rare complication of cyclosporine is blindness, which may be irreversible

Cyclophosphamide: Immediate: tingling and metallic taste, nausea and vomiting, ADH-like effect, cardiotoxic at high doses (>70 mg/kg). Rare - pulmonary toxicity, urticaria and flushing, mucositis Delayed: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility

Fludarabine*:* Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, and rarely hemolysis and pulmonary toxicity, hemolytic anemia and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.

Methotrexate: Mucositis, nausea, dizziness, neutropenia, thrombocytopenia, malaise, fatigue, fever, melena, headaches, blurred vision, rashes, alopecia, elevated liver functions,

Hematologic: Myelosupression [leukopenia (nadir 7 days) thrombocytopenia, anemia]

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

Urogenital: Severe nephropathy or renal failure, azotemia, cystitis, hematuria; defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction and vaginal discharge; infertility, abortion, fetal defects. With high doses of methotrexate, close attention to renal function including adequate hydration, and urine alkanization are essential for safe administration.

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

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

Skin: Erythematous rashes, pruritis, urticaria, photosensistivty, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis.

Central Nervous System: Headaches, drowsiness, blurred vision. There have been reports of leukoencephalopathy following intravenous administration of methotrexate to patients who have craniospinal irradiation. Aphasia, hemiparesis, paresis, and convulsions have also occurred following administration of methotrexate. Following low doses, occasional patients reported transient subtle cognitive dysfunction, mood alteration, or unusual cranial sensations.

Other: Opportunistic infections, arthralgia/myalgia, loss of libido/impotence, diabetes, and osteoporosis. A few cases of anaphylactoid reactions have been reported.

Antimicrobials in general: Allergic reactions, renal impairment (gentamicin, vancomycin, amphotericin, acyclovir), "red man" syndrome -vancomycin, hepatic damage (acyclovir, rifampicin)

15.3.2 The donor

Related to filgrastim (G-CSF): G-CSF has been given to large numbers of normal donors without major side effects or long term consequences. The immediate side effects of G-CSF are bone pain, fatigue, insomnia, myalgia, headache, chills, low grade fever, rash, injection site irritation, exacerbation of pre existing inflammatory conditions, splenomegaly, reversible elevation in uric acid, LDH, and leucocyte alkaline phosphates. These are usually mild and are self limiting. Reversible thrombocytopenia, with platelet counts falling to the region of 100,000/cu mm is frequent. G-CSF administration could result in splenic rupture, a potentially life-threatening complication. Up to now five cases of splenic rupture have been reported in healthy donors (Becker et al. 1997, Falzetti et al. 1999, Balaguer et al. 2004, Dincer et al. 2004, Nuamah et al. 2006). Donors will be asked to avoid vigorous activities and to report any left upper abdominal and/or shoulder pain to the research team or the on-call physician for the NIH Department of Transfusion Medicine at 301-496-1211**.**

Patients with ongoing ischemic heart disease have been reported to have angina seemingly temporally related to G-CSF administration and apheresis. The discomfort from G-CSF mobilization and leukapheresis for collection of blood stem cells are probably lower than those associated with marrow harvesting.

Related to Central line placement: It is estimated that about 50% of the donors will require intravenous central line placement to successfully complete apheresis. Intravenous line placement in the femoral vein using a temporary double-lumen Arrow catheter carries a small risk of bleeding, bruising or pain and a very low risk of accidental injury to the adjacent artery and nerve. Some patients may experience a vasovageal reaction (lightheadedness, or rarely, fainting due to temporary lowering of blood pressure). These risks are minimized by using only trained experienced MICU staff for the procedure.

Related to Leukapheresis: Adverse reactions related to leukapheresis include hypotension resulting from transient blood volume loss and cutaneous paresthesia from the use of anticoagulant. The former toxicity can be corrected by postural changes and volume replacement. The latter is manageable with slowing the rate of anticoagulant infusion and/or providing calcium supplement. In exceptional instances the donor may be required to donate PBPC a third time or to give bone marrow. Donation of PBPC on three successive days significantly increases the risk of thrombocytopenia (< 1 00,000/ul). However, thrombocytopenia is transient and unlikely to cause clinical sequelae. There is no additional risk to the donor giving marrow after PBPC donation (than would normally be associated with bone marrow harvesting).

15.4 Risks in relation to Benefit

This protocol was closed to accrual and follow up in June 2008; all subjects are off study and this protocol is in data analysis phase only therefore, the research currently does not involve greater than minimal risk.

15.4.1 For Adult Transplant Subjects

Clinically, the approach is ethically acceptable because we are targeting a patient group with incurable neoplasms, which have become refractory to conventional therapy. The protocol also aims to decrease the risk of transplant-related mortality, thus making patients with advanced metastatic disease candidates for this therapy. The risk of death due to complication from the allogeneic BMT procedure is justified by the anticipated benefit of potentially eradicating the subject's underlying metastatic disease and the relation of the anticipated benefit to the risk is at least as favorable to the subjects as presented by available non-transplantation therapies.

Therefore, for adult transplant recipients on this protocol, the research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102).

15.4.2 For Pediatric Transplant Subjects

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.405 as follows:

(a) *the risk is justified by the anticipated benefit to the subjects*: Clinically the approach is ethically acceptable because we are offering children ≥10 years of age with incurable metastatic neoplasms, which have become refractory to conventional therapy an alternative to palliative care.

(b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches. The protocol aims to decrease the risk of transplant-related mortality, thus making more patients candidates for potentially curative therapy and
(c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore for pediatric transplant recipients on this protocol, the research involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects.

15.4.3 For Adult Donors

Normal identical or single HLA locus mismatched family members will be co-enrolled into this study as stem cell donors. The stem cell collection aspect of this protocol is not investigational. Despite the risks associated with this procedure (section 15.3), potential benefit does exist for family donors. The donor derives psychosocial benefit from donating stem cells both at the time of donation and possibly into the future, especially in view of the reduced life expectancy due to this disease in a family member. Other potential benefits include detection of illnesses, determination of blood cell counts, and evaluation of kidney and liver function in the potential donor at the time of screening.

Therefore, for adults participating as stem cell donors, the research involves a greater than minimal risk but presents the prospect of direct benefit to the individual subject (45 CFR 46.102).

15.4.4 For Pediatric Donors

The inclusion of children as donors satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.405 as follows:

(a) *the risk is justified by the anticipated benefit to the subjects*: Despite the risks associated with this procedure, it is standard clinical practice to include children as donors for allogeneic peripheral blood stem cell transplantation. Increased risk is due to G-CSF administration and central line placement. Leukapheresis in children, as discussed under 15.2, is safe and effective both in our experience and in others', and there have never been any leukapheresis-related deaths in the history of the Transfusion Medicine department at NIH.

(b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches. As with adults the pediatric donor derives psychosocial benefit from donating stem cells especially in view of the reduced life expectancy due to this disease in a family member. Other potential benefits include detection of illnesses, determination of blood cell count and evaluation of kidney and liver function in the potential donor at the time of screening.

(c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore for children participating as stem cell donors the research involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects.

15.4.5 For Pediatric donors – healthy volunteers- involved in laboratory research studies.

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.404 as follows:

(a) *The research does not involve greater than minimal risk*. Blood and bone marrow specimens for research are obtained concurrently with clinically indicated sampling. Therefore, there is no risk associated with sample collection for research because research will only performed on material obtained during standard clinical intervention.

Research specimens will be stored in the Dr. Childs's laboratory. Samples will never be labeled with the child's name. Samples will be assigned a unique code known only to the PI, which will serve as a link to the child's clinical information collected as part of this research protocol. No samples will be provided to investigators outside the branch. Therefore confidentiality is protected.

Only those laboratory tests approved by the IRB and involving not greater than minimal risk will be conducted (See Appendix D laboratory studies D1-D6 are determined to be greater than minimal risk). Research will not include genetic testing. Therefore, there is no genetic testing associated risks.

(b) adequate provisions is made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore, for *Pediatric donors – healthy volunteers- participating in laboratory research studies* on this protocol, the research does not involve greater than minimal risk (45 CFR 46.404).

15.5 Informed Consent Process and Procedures

The investigational nature and research objectives of this trial, the procedures and attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study. Drs. Childs, Srinivasan, Barrett, Young will lead this discussion.

If the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

If the donor is a minor, assent will not be sought until an evaluation by a social worker and mental health specialist (psychologist or psychiatrist) is completed to determine the minor donor's willingness to participate. As detailed above, the parent who signs the consent for the minor must be a legally recognized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor donor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent

When the assent is not age appropriate, the study will be explained to the child and the assent will be obtained verbally from the child.

Re-Consent for Minors when they reach the age of majority: When a pediatric subject reaches age 18, continued participation will require re-consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued

use of data or samples collected during their prior visit. Given the length of time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts to contact them, we are unable to contact.

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 a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could 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.

At any time during participation in the protocol that new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

15.6 CONFLICT OF INTEREST

The PI assured that each AI listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team reported a potential conflict of interest.

This protocol has no associated patents, CRADAs, or CTAs. An MTA has been executed and a copy is on file with the Office of Clinical Affairs for the transfer of sample to Pierre Ronco, MD, PhD, Head, Nephrology Department, Unit 702 At Tenon Hospital, Paris, France tel : + 33 1 56 01 66 39/38 pierre.ronco@tnn.aphp.fr

16.0 PHARMACEUTICALS

16.1 ANTITHYMOCYTE GLOBULIN (Atgam)

Other: Antithymocyte Gammaglobulin, Antithymocyte Globulin, Atgam, Antithymocyte Immunoglobulin.

Compound: Principally monomeric IgG, prepared from plasma or serum of healthy horses hyper-immunized with human thymus lymphocytes.

Action: Immunosuppressive agent. Exact mechanism of immunosuppression of ATgam has not been fully elucidated but may involve elimination of antigen-reactive T-cells in peripheral blood and/or alteration of T-cell function.

Side effects: see section 15.3, Risks and discomforts.

16.2 CYCLOPHOSPHAMIDE

Generic: cyclophosphamide
other: Endoxan, Cytoxan, Neosar, NSC#: 26271
Chemical: 2-bix (2-chloroethyl) amino tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide monohydrate.
Classification: Alkylating agent
Action: Prevents cell division by altering DNA
Metabolism: Converted in the liver by microsomal enzymes to active nitrogen mustard like compounds.Excreted by the kidney as metabolites and unconverted cyclophosphamide.
Availability: 100, 200, and 500 mg vials. Commercial: Meade Johnson and Adria.
Storage: Stable at room temperature. After reconstitution use within 24 hours. Administration: Dissolved in 250 ml D5W and administered over one hour.
Precautions: Institute antiemetic therapy with a serotonin antagonist before administration and continue with anti-emesis for 24 hours following administration.
To prevent hemorrhagic cystitis: give mesna prior to cyclophosphamide. Continue with mesna following cyclophosphamide; IV hydration as per Appendix A.
Side effects: See section 15.3, Risks and discomforts.

16.3 CYCLOSPORINE (CSA)

Dose:1.5 mg/kg/dose BID IV will begin on day -4 through day 100. Dosing will be based on actual body weight unless the patient is "obese" (BMI > 35) when practical weight will be used. Switch to the equivalent oral CsA dose divided bid on day 14 or when able to tolerate PO.
Levels: Serum levels monitored weekly
Mechanism of Action: Blocks T cell activation by inhibiting transport of cytoplasmic component of Nuclear factor of activated T-cells (NFAT) into nucleus, thereby preventing IL-2 transcription.
Metabolism: CSA is metabolized primarily in liver but predominate toxicity is renal.

Dose Modifications

Renal insufficiency
- Creatinine >2.0 mg/dl reduce dose 25%
- Creatinine >3.0 mg/dl reduce dose 75%
- Creatinine >4.0 mg/dl hold

Hepatic insufficiency
- If increase in bilirubin or transaminases >100% over baseline decrease by 2mg/kg/d

Serum levels
- Adjust dose for therapeutic range (200-400ug/ml)

Side effects: See section 15.3, Risks and discomforts.

16.4 FLUDARABINE PHOSPHATE

Generic: fludarabine phosphate
Other: Fludara, Berlex
Chemical: 2-fluoro-ara-monophosphate
Classification: Fluorinated nucleotide analog
Action: Inhibits DNA synthesis
Metabolism: Converted intracellularly to the active metabolite 2-fluoro-ara-ATP.
Availability: 50 mg for iv use only. Commercial: Biospherics
Side effects: See section 15.3, Risks and discomforts.

16.5 G-CSF (Human recombinant granulocyte colony stimulating factor)

G-CSF (Amgen), Filgrastim
Classification: glucoprotein
Action: Regulates the production of neutrophils from bone marrow progenitor cells and mobilizes primitive hematopoietic stem cells from the bone marrow into the circulation.
Metabolism: Absorption and clearance of G-CSF follow first order kinetics. A positive linear correlation occurs between the parenteral dose and both the serum concentration and area under the concentration-time curves. The elimination T1/2 is 3.5 hours.
Administration: G-CSF may be given sc a single daily dose or iv over 30 min.

G-CSF is a protein that is produced by normal human bone marrow cells. G-CSF used in this study is purified from the product of E. coli with recombinant DNA technology. The only differences between this product and natural G-CSF are an additional N-terminal methionine necessary for expression in E. coli and the fact that this product is unglycosylated. G-CSF is approved by the US Food and Drug Administration to be used in preventing chemotherapy-induced leukopenia. This drug has been used in cancer patients for prolonged periods of time without major toxicities. The extensive use of this drug in many patients also proved its safety at a dose of 10 _g/kg/day. The most frequent side effect is bone pain originating from expansion of cells inside the bone marrow. It can affect up to 80% of the subjects at this dosage range. The pain is usually mild and can be alleviated most of the times by acetaminophen or other non-steroidal analgesics and will cease altogether once G-CSF is discontinued. G-CSF is associated with increased inflammation in patients with autoimmune diseases or subjects with infection, however these patients are not eligible for this study. These are readily reversible after cessation of the drug. Leukocytosis is expected after administration of G-CSF. Other adverse reactions associated with the use of G-CSF as cited in the literature include: fatigue (50%), headache (30%), muscle aches (25%), insomnia (rare), fever (rare), transient but reversible increases of alkaline phosphatase, lactate dehydrogenase, and uric acid levels, exacerbation of preexisting skin rashes, and thrombocytopenia (mild, reversible).

Availability: Recombinant G-CSF is available from the pharmacy at the National Heart, Lung and Blood Institute in colorless glass, single-use vials containing either 300_g in 1.0 ml vials or 480_g in 1.6 ml vials (300 _g/ml). It is formulated as a sterile, clear, colorless liquid in a 10 mm sodium acetate buffer at pH 4.0. The quantitative composition (per ml) is:

Recombinant G-CSF	300 _g
Acetate	0.59 mg
Mannitol	50 mg
Tween 80™	0.004%
Sodium	0.035 mg
Water for injection (qs ad) to 1.0 ml	

Storage: The intact vials of G-CSF should be stored under refrigeration (2 - 8°C).
Stability: G-CSF in the intact vial is stable for 36 months when stored in a refrigerator at 2 - 8°C. A single brief exposure (up to 7 days) to elevated temperatures (< 37°C) does not affect the stability. G-CSF should not be frozen, and vials, which have been frozen should not be used.
Side effects: See section 15.3 Risks and Discomforts.

16.6 METHOTREXATE

Generic: Methotrexate sodium injection
Other: Rheumatrex® (Methotrexate)
Classification: Antimetabolite:Immunosuppressive agent

Dose: 5 mg/m^2 intravenously to be given on post-transplant days +1, +3, and +6. Methotrexate will be held if the serum creatinine is > 3mg/dl. Patients with elevated liver function tests (i.e. transaminases) greater than 3 times the upper limit of normal may have methotrexate held at the discretion of the principal investigator.

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

Administration: Methotrexate Sodium for Injection, freeze dried, preservative free, low sodium, for single use only, is available in 20 mg, 50 mg, and 1 gram vials, containing approximately 0.14, 0.33, and 7 meq of sodium respectively. Reconstitute immediately prior to use with an appropriate sterile, preservative-free medium.

Side effects: See section 15.3, Risks and Discomforts

16.7 MYCOPHENOLATE MOFETIL (MMF)

Generic: Mycophenolate mofetil

Other: CellCept_, Roche Laboratories

Classification: Immunosuppressive agent

Dose: 1 gram p.o. twice daily given to some patients with steroid resistant acute GVHD or steroid dependent chronic GVHD.

Action: Mycophenolic acid produces potent, noncompetitive inhibition of inosine monophosphate dehydrogenase, thus blocking de novo synthesis of guanosine nucleotides; as lymphocytes depend upon the de novo pathway for purine synthesis, lymphocyte proliferation is inhibited. In vitro and in vivo studies have demonstrated the ability of mycophenolic acid to block proliferative responses of T and B lymphocytes, and inhibit antibody formation and the generation of cytotoxic T-cells.

Metabolism: Mycophenolate mofetil is rapidly converted to it's active metabolite, mycophenolic acid in the liver. Mycophenolic acid is subsequently metabolized to mycophenolic acid glucuronide which is inactive and is excreted via the urine and bile. Enterohepatic recirculation of mycophenolic acid may occur. The elimination half-life of mycophenolic acid is approximately 11 to 18 hours.

Side effects: See section 15.3, Risks and Discomforts.

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APPENDIX A: PREPARATIVE REGIMEN

DOSE LEVEL I
Cyclophosphamide regimen

	Hour	
Day -7	0	At midnight Hydrate: NS 2.6ml/kg/hr 10 mEq/l KCl (starting 12 hours pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion)
	11	Ondansetron 8 mg IV q 12 hrs or an equivalent serotonin antagonist + decadron 8 mg PO q12 hrs x 1 day. Mesna (per PBSCT Supportive Care Guidelines) Furosemide 20 mg iv.
	12	Cyclophosphamide 60 mg/kg IV in 250 ml D_5 in 1 hr.
	13	Mesna (per Supportive Care Guidelines)
Day - 6	11	Ondansetron 8 mg IV q 12 hrs or an equivalent serotonin antagonist + decadron 8 mg PO q12 hrs x 1 day. Mesna (per Supportive Care Guidelines) Furosemide 20 mg iv
	12	Cyclophosphamide 60 mg/kg IV in 250 ml D_5 IV over 1 hr
	13	Mesna (per Supportive Care Guidelines)
Day -5		Stop IV hydration (24 hours after last cyclophosphamide dose) Monitor electrolytes and fluid balance while receiving hydration.

Day -5 to Day -1
Hour 9 Fludarabine 25 mg/m^2 IVBP over 30 minutes for 5 days

DOSE LEVEL II Same as dose level I except patients will receive ATgam at a dose of 40 mg/kg/d for 4 consecutive days on day -5 to day -2.

APPENDIX B: ATG DESENSITIZATION

An intravenous line must be in place, and emergency medications (diphenhydramine 50 mg, epinephrine 1:1000, hydrocortisone 100mg) should be at the bedside prior to initiating desensitization. Desensitization should be performed in a limb where a tourniquet can be proximally placed if a reaction occurs. Injections are given intravenously every 15 minutes according to the following timetable. If an injection produces a large local reaction, the same dose is repeated before progressing to the next larger dose. Vital signs should be recorded every 15 minutes.

Dose #	Concentration of ATG (mg/ml)	Volume/Dose	Rate	Cumulative Dose (microg)
1	0.0001	1 ml/0.1 mcg	Push	0.1
2	0.0005	1 ml/0.5 mcg	Push	0.6
3	0.002	1 ml/2 mcg	Push	2.6
4	0.005	1 ml/5 mcg	Push	7.6
5	0.01	1 ml/10 mcg	Push	17.6
6	0.01	3 ml/30 mcg	Push	47.6
7	0.01	10 ml/100 mcg	Push	147.6
8	0.01	160 ml/1600 mcg	16 ml/min for 10 min (total 160ml)	1747.6
9	Therapeutic Infusion			

APPENDIX C: SERIOUS ADVERSE EVENT REPORT FORM (As July 1, 2019 this form no longer being used)

Definition of a serious adverse event: For the purposes of this form, a serious adverse event is any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or in the opinion of the investigator represents other significant hazards or potentially serious harm to research subjects or others. A serious adverse event is considered unexpected if it is not described in the Package Insert or in the Investigator's Brochure (for FOA investigational agents), in the protocol, or in the informed consent document.

Please complete the information requested below and forward it to the NHLBI IRB via the NHLBI Office of Clinical Affairs (OCA), Melissa Bryant, CRC 4-1581, Room 4-1580 with a copy to Dr. Richard Cannon, Clinical Director, Clinical Research (CRC-4-1581, as soon as possible, but no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events. In addition, continue to follow FDA and the NIH Office of Biotechnology Activities (OBA) reporting requirements if your research involves an IND / IDE or gene transfer.

1. Protocol number: 99-H-0064

2. Protocol title: Exploratory study of non-myeloablative allogeneic stem cell transplantation and donor lymphocyte infusions for metastatic neoplasms refractory to standard therapy

3. Principal Investigator: Richard W. Childs, M.D.
   Institute: NHLBI/HB
   Office: Bldg 10, Room CRC 3-5332
   Phone: 301-594-8008
   FAX:
   E-mail: childsr@NHLBI.NIH.gov

4. a. Date of serious adverse event:

b. Date the Sponsor/Principal Investigator were first made aware of this event. (please note that it is the investigators responsibility to report this event within the stipulated time frame once they are made aware of the event)

c. If there is significant lag time from the date the event occurred to the date the event was reported, please provide a brief explanation.

5. Location of serious adverse event (e.g., at NIH or elsewhere):

6. Was this an unexpected adverse event? Yes [ ] No [ ]

7. Brief description of subject(s) (do not include identifiers) Sex: Age: Diagnosis:

8. Brief description of the nature of the serious adverse event (attach description separately if more space needed):

9. Category (outcome) of the serious adverse event:

1. [ ] death
2. [ ] life-threatening
3. [ ] hospitalization-initial or prolonged
4. [ ] disability / incapacity
5. [ ] congenital anomaly / birth defect
6. [ ] required intervention to prevent permanent impairment
7. [ ] other

10. Relationship of Serious Adverse Event to research:

[ ] 1 = Unrelated (clearly not related to the research)
[ ] 2 = Unlikely (doubtfully related to the research)
[ ] 3 = Possible (may be related to the research)
[ ] 4 = Probable (likely related to the research)
[ ] 5 = Definite (clearly related to the research) .

11. Have similar adverse events occurred on this protocol? Yes [ ] No [ ]

If "Yes", how many? _____ Please describe.

12. What steps do you plan to take as a result of the adverse event reported above? Provide documentation to the IRB for review and approval of any of the steps checked below.

[ ] no action required
[ ] amend protocol
[ ] amend consent document
[ ] inform current subjects
[ ] terminate or suspend protocol
[ ] other (describe)

Signature of Principal Investigator:_____ Date:_____

APPENDIX D: APPROVED LIST OF NHLBI LABORATORY STUDIES

IRB APPROVED NHLBI CMTB LABORATORY RESEARCH STUDIES v. 2.5.2013

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
A	**Stem Cell Allotransplantation Section (Dr. A. John Barrett) – no longer active**		
A.1	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
A.2	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
A.3	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
A.4	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies.	No	No
A.5	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
A.6	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
A.7	Identification of individual T cell clones by their T cell receptor sequence.	No	No
A.8	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA,protein, or peptide expression in cells or fluids.	No	No
A.9	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No
A.10	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
A.11	Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions.	No	No
B	**Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)**		
B.1	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No

B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No
C.5	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
C.6	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
C.7	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
C.8	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
C.9	Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No

C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2*.	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No

E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Lasar capture microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No