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CLINICAL RESEARCH PROJECT

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Title: A Pilot Study of Early Treatment of Acute Graft Versus Host Disease with Bone Marrow-Derived Mesenchymal Stem Cells and Corticosteroids: Correlation of Disease Severity and Response with Biomarkers

Other identifying words: BMSC, corticosteroid, GVHD, immunotherapy, adoptive cellular therapy

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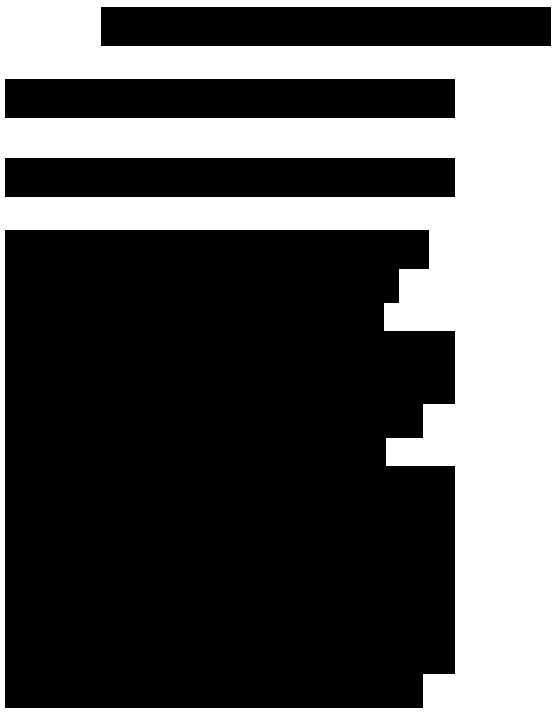

**Investigators designated to obtain informed consent from research subjects.*

Subjects of Study:	Number	Sex	Age range
Subjects:	up to 25	either	age ≥ 4 years

Project involves ionizing radiation?	Yes (Medically Indicated Only)
Off site project?	No
Multi-institutional project?	No
DSMB Involved?	Yes

PRECIS

This is a pilot study evaluating the addition of bone marrow-derived mesenchymal stem cell (BMSC) infusions to corticosteroids for the early treatment of acute graft versus host disease (GVHD). Acute GVHD is a major complication following allogeneic stem cell transplant. GVHD occurs when T-lymphocytes in the donor graft respond to signals from recipient cells and cause tissue damage. This process can lead to organ injury, increased risk of infection, and graft failure. Corticosteroids have been used as the primary therapy for acute GVHD for decades, and guidelines currently recommend their use as front line treatment. Recent prospective data from the Blood and Marrow Transplant Clinical Trials Network shows that GVHD will be cured in about half of patients with steroids alone. Patients who do not respond to steroids are considered steroid-resistant, and this is associated with much worse survival. It is possible to predict which patients will go on to have steroid-resistant GVHD by measuring the plasma concentration of the molecule called suppression of tumorigenicity 2 (ST2). BMSC infusions have been used to treat steroid-resistant acute GVHD successfully, but despite a track record of safety, little is known about the use of BMSC in the early treatment setting. The main objective of this study is to explore the feasibility of administering BMSC within 5 days of diagnosis of acute GVHD. Our study will for the first time use the ST2 biomarker to more accurately assign acute GVHD to steroid refractory or sensitive, and explore changes in ST2 and other biological markers of BMSC function and their correlation with clinical response. In the process of this study, we will assess the safety and feasibility of early treatment according to our regimen, obtain estimates of efficacy at important GVHD therapy time points, and determine if treatment with BMSC can prevent the progression of GVHD in patients with high risk of GVHD progression as measured by biomarkers.

The Cell Processing Section of the Department of Transfusion Medicine at the Clinical Center NIH has developed a BMSC repository at NIH. The NIH BMSC are a third party, early passage product based on the EU manufacturing approach. The NIH BMSC cellular product was administered safely to transplant recipients with steroid-resistant acute GVHD in a phase I study (protocol 12-H-0010, IND #14596) conducted from March 2012 to October 2012 at NIH. This pilot study is a continuation of the previous study and open to allogeneic stem cell transplantation recipients at NIH (age ≥4 yrs) with *de novo* acute GVHD requiring systemic therapy, either directly after allogeneic transplantation or following treatment with donor lymphocyte infusion. Subjects will receive BMSC infusions (target dose of 2×10^6 BMSC/kg for up to 12 doses) in addition to standard upfront therapy with corticosteroids. The primary endpoint will be the proportion of patients without a treatment-related severe adverse event (TRSAE) at day +56. Responses will be assessed at day +28 and +56 from the initial diagnosis. Responses will be correlated to changes in GVHD biomarkers including ST2, Reg3a, TNFR1, and IL-6. Subjects will be enrolled at first diagnosis of acute GVHD, and the first BMSC infusion will be given within 120 hours of the first dose of corticosteroids. BMSC infusions will be given twice weekly for the first 4 weeks. Subjects with a complete response at the end of week 4 will not receive further infusions. All other subjects will receive BMSC infusions weekly for four additional weeks. Safety will be monitored continuously with a stopping rule for toxicity based on the treatment-related serious adverse event rate. Research samples will be drawn at regular intervals to explore biological correlates of response and to investigate the mechanism of action of BMSC.

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1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

Determine the safety and feasibility of adding BMSC infusions to corticosteroid therapy in allogeneic stem cell transplant recipients within 5 days of the diagnosis of *de novo* acute GVHD requiring treatment with systemic corticosteroids.

1.1.2 Secondary Objectives

Estimate the efficacy of our proposed regimen at well-established GVHD response time points and correlate responses to plasma GVHD biomarkers of disease severity (ST2) and response (Reg3a, TNFR1, IL-6).

2. BACKGROUND

2.1 ACUTE GRAFT-VERSUS-HOST DISEASE

Acute GVHD is an inflammatory process that occurs in the majority of patients who receive an allogeneic stem cell transplant. GVHD occurs when host antigen-presenting cells (APC) activate in response to signals from damaged tissue or pathogens. APC present antigens to donor T-lymphocytes, leading to alloreactivity, cytokine release, and increasing tissue destruction, reviewed in[1]. Of the more than 7,000 patients who undergo allogeneic stem cell transplant in the US each year[2], approximately 35-50% will develop grade II-IV acute GVHD despite prophylactic immunosuppression[3]. GVHD is the second leading cause of death in patients undergoing transplant and it is the leading cause of non-relapse mortality[2]. The standard initial therapy for acute GVHD is high-dose intravenous corticosteroids[4]. The response rate to single-agent corticosteroid therapy, when analyzed in large retrospective reviews, is approximately 50%[5]. In a recent randomized prospective study comparing mycophenolate mofetil plus steroids and steroids alone, the day 56 acute GVHD survival was 50.4% in the steroids alone arm (n=119)[6]. Mortality in patients with acute GVHD is greatest among those who fail to achieve a complete response with initial treatment[5,7]. Although steroid-resistant acute GVHD can be treated with anti-lymphocyte globulin or a variety of monoclonal antibodies, there is no standard treatment approach and steroid-resistant GVHD has a one year mortality between 50-80%[8]. Acute GVHD usually occurs in the first 100 days of allogeneic stem cell transplant and may involve the skin and/or gastrointestinal tract and/or liver. Following is a summary of the major manifestations of acute GVHD.

2.1.1 Skin GVHD

Skin involvement is usually the first sign of GVHD. It varies in extent from a rash affecting extensor surfaces face and hands to a generalized erythroderma affecting the entire body. Severity varies according to the degree of epidermal damage from a mild desquamation to bullae formation and separation of the dermo-epidermal junction. Skin GVHD is usually well controlled with steroids but severe extensive skin GVHD carries mortality from secondary infection.

2.1.2 Gastrointestinal GVHD

Acute gastrointestinal GVHD can affect the entire digestive tract leading to diarrhea, blood loss, vomiting, nutritional failure, weight loss and secondary infection. Lymphocyte infiltration in the submucosa is associated with crypt damage and loss with characteristic single-cell apoptosis in the columnar epithelium leading to complete loss of the epithelium.

2.1.3 Hepatic GVHD

Hepatic GVHD primarily affects the bile canaliculi with peri-canalicular lymphocyte infiltration, and damage to biliary endothelium. Parenchymal tissue is secondarily affected giving rise to the typical mixed obstructive/hepatitis pattern of LFTs. While hepatic GVHD is usually mild to moderate and responds to immunosuppression, some patients develop worsening, life-threatening hepatic GVHD with a "vanishing bile duct" histological picture of rising bilirubin and worsening liver function. This usually but not always occurs in association with severe gut GVHD.

2.2 PRIMARY THERAPY FOR ACUTE GVHD

Corticosteroids have been used as the first line treatment for acute GVHD for decades. Several agents have been added to upfront steroids in clinical trials in order to improve response rates, most commonly: ATG[9-11], daclizumab[12,13], denileukin difitox[14], and etanercept[15]. No agent or combination of agents has been proven to be more effective than steroids alone. Recently the Blood and Marrow Transplant Clinical Trials Network (BMT-CTN) conducted two randomized trials (BMT CTN 0302 and BMT CTN 0802) focused on improving the initial response rates of *de novo* acute GVHD to therapy.

2.2.1 BMT CTN 0302

The first trial was a randomized, four-arm, phase II trial designed to identify the most promising agent to combine with steroids in a phase III trial for upfront treatment of acute GVHD[16]. Patients (n=180) were accrued from August 2005 to March 2008 and randomized to receive either etanercept, mycophenolate mofetil (MMF), denileukin difitox, or pentostatin in combination with standard doses of corticosteroids for initial therapy. The proportion of complete responses at day +28 were etanercept (26%), MMF (60%), denileukin difitox (53%), and pentostatin (38%). Day 56 ORR rates were 59%, 78%, 68%, 71% respectively, and OS at 6 months was 59%, 71%, 63%, and 55% respectively. Infection rates and overall toxicities were lower in the etanercept and MMF arms.

2.2.2 BMT CTN 0802

Based on the results of BMT CTN 0302, a phase III, multi-center randomized trial (BMT CTN 0802) was conducted comparing corticosteroids/placebo to corticosteroids/MMF[6,17]. Patients received prednisone at 2mg/kg/day with either MMF (1000mg po/IV q8h) or placebo. MMF was continued until day +56 or until steroid discontinuation if sooner. The proportion of GVHD free survival at day +56 after randomization was MMF 59.5% and placebo 50.4% (p=0.16). Overall survival, non-relapse mortality, and incidence of infection were similar in both arms. The study was stopped at a predetermined interim analysis when it was shown that the addition of MMF was unlikely to improve clinical outcomes when compared to steroids alone.

Based on the findings of these recent well-designed multicenter studies, corticosteroid therapy remains the only standard treatment approach for *de novo* acute GVHD requiring systemic therapy.

2.3 BIOLOGY OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Bone marrow derived mesenchymal stem cells (BMSC) are multi-potent bone marrow cells able to differentiate *in vitro* and *in vivo* into tissues of mesenchymal origin[18]. BMSC support growth and differentiation of hematopoietic progenitor cells in bone marrow microenvironments and in animal models, promote engraftment of hematopoietic cells[19,20]. Infused BMSC home to sites of tissue injury in mice and non-human primates and secrete bioactive molecules[21-23]. BMSC are immune-modulatory and anti-inflammatory[24,25]. BMSC do not induce proliferation or interferon-γ production, or upregulation of activation markers on allogeneic lymphocytes[26]. BMSC suppress proliferation of activated lymphocytes

in vitro in a dose-dependent, non-HLA-restricted manner[27]. In a baboon skin-graft model infusion of *ex-vivo* expanded BMSC prolonged the time to rejection of histoincompatible skin grafts. Suppression of skin graft rejection was effective whether BMSC came from the same donor as the stimulator lymphocytes, the same donor as the responder lymphocytes, or from an unrelated (third-party) donor[21]. Furthermore, BMSC also block established lymphocyte responses. Addition of BMSC four days after the initial mixing and stimulation of lymphocytes led to a suppression of proliferation comparable to that seen when BMSC were present from the onset of the mixed lymphocyte reaction culture[28]. Infused BMSC do not induce immunological memory in the recipient even when they are completely mismatched with the recipient[28]. Thus there is specific tolerance to BMSC infusions which allows BMSC of any source to be given to recipients without being rapidly rejected. BMSC promote tissue repair. Infused BMSC improve the outcome of acute renal, neural, and lung injury, possibly by promoting a shift from production of pro-inflammatory cytokines to anti-inflammatory cytokines at the site of injury. BMSC promote healing after radiation injury in experimental animals[29-31].

In man, BMSC can be isolated from bone marrow, cultured *ex vivo*, and expanded many fold[32]. Culture expanded BMSC represent a homogeneous population by flow-cytometric measures of cell-surface markers whose minimal criteria have been defined as positive for CD146, CD90, HLA class I and negative for hematopoietic cell markers[33,34]. The ease with which BMSC can be cultured from human material, their unique properties of immunomodulation and tissue repair, and their safety profile to date have made the therapeutic infusion BMSC a rapidly developing area of adoptive cellular therapy[35,36]. Because of this, NIH established a repository for BMSC in the Department of Transfusion Medicine Cell Processing Service[37,38] and conducted a phase I trial exploring the safety and efficacy of the NIH BMSC product in patients with steroid-resistant acute GVHD and tissue injury post-allogeneic stem cell transplant[39].

2.4 Clinical Experience With BMSC in GVHD

BMSC infusions have been used extensively in early phase studies for the prevention and treatment of GVHD.

2.4.1 GVHD Prophylaxis

The administration of BMSC for GVHD prophylaxis at the time of stem cell infusion or at the time of engraftment has been studied in several early stage trials in both pediatric and adult populations. Altogether just over one hundred patients in more than 10 cohorts have been given BMSC early in the course of allogeneic stem cell transplant[40-50]. Patients commonly received doses of 1-2x10^6 BMSC/kg, but as much as 5x10^6 BMSC/kg, in one infusion. In the majority of these studies treatment with BMSC was considered safe and GVHD rates compared favorably to historical control groups. One study[46] reported an increase in the risk of leukemic relapse in a cohort of patients treated with BMSC at the time of stem cell infusion to aid engraftment. In this trial conducted by Ning et al. in China, patients with hematologic malignancy (n=10) were co-transplanted with BMSC, and a non-BMSC group was followed for comparison (n=15). Grades II-IV acute graft-versus-host disease (GVHD) was observed respectively, in one patient (11.1%) in the BMSC group, and eight (53.3%) patients in the non-BMSC group. The number of patients who relapsed were six (60.0%) and three (20.0%), and the 3-year disease-free survivals were 30.0 and 66.7%, respectively. This study has been criticized for methodological flaws[51] and no link has been established between BMSC treatment and leukemic relapse in a long term follow up of a larger cohort[52] and in one meta-analysis inclusive of 8 randomized trials (including the aforementioned study by Ning et al.)[53].

2.4.2 *De novo* GVHD

Kebriaei *et al.* conducted the first prospective trial of third-party BMSC for the treatment of *de novo* acute GVHD using a pre-manufactured, universal donor formulation of BMSC (Prochymal®)[54]. This industry sponsored, randomized, multicenter trial compared two different dose levels of BMSC (2 or $8x10^6$ BMSC/kg) combined with standard corticosteroid therapy. Patients were infused with BMSC within 72 hours of acute GVHD diagnosis and they received one additional dose 3 days later. Of 31 adult patients, 71% had a CR at day 28 after therapy (primary endpoint), and the initial ORR was 94%. No evidence of a dose response relationship was seen. These promising results led to the use of Prochymal® in a large industry-sponsored phase III trial for *de novo* acute GVHD (Protocol 265, Osiris Therapeutics, Inc.). The primary endpoint was CR within 28 days of treatment administration, and treatment failures included patients without CR at 28 days and those requiring increased doses of corticosteroids or additional immunosuppressive therapy. This study failed to reach significance in the primary endpoint, and the results have not been published[55]. The higher than historically reported response to steroid therapy, the use of late passage BMSC, the predominance of skin GVHD, and the choice of primary endpoint at day 28 are widely attributed to have been the cause for failure to meet the primary endpoint[56,57].

2.4.3 Refractory GVHD

By far the largest number of patients have treated with BMSC infusions have had steroid-resistant GVHD. Below is a summary of over one decade of trials for BMSC in steroid-resistant GVHD. Response rates are promising for a disease with high morbidity and mortality, but it is difficult to draw comparisons between studies due to their small size, heterogeneous design, and use of different outcome measures.

Year published	First author, country	Patient numbers	Donor, BMSC source	GVHD Grade	Passage	BMSC dose (/kg), dose range, number of infusions, number range	Response	Comments
2004	Le Blanc *et al.*[58], Sweden.	1	Haplo, BM	IV	Early	Two infusions- first 2x10e6, second 1x10e6 at relapse	1 CR (100%)	First use of BMSC in acute GVHD, patient age 9, CR with first infusion then relapse, CR with second infusion
2006	Ringden *et al.*[59], Sweden	8	MRD, haplo, third party, BM	III-IV	Early	1x10e6, 0.7-9x10e6, 1-2 doses, dosed weekly	6 CR (75%)	ORR 75%., no side effects or infusion toxicity.
2007	Fang *et al.*[60] and [61], China	6	Haplo, third party, adipose-derived BMSC	III-IV	Early	1x10e6 x 1 dose	5 CR (83%)	First clinical study of adipose-derived BMSC for the treatment of GVHD.
2008	Le Blanc *et al.*[62], Sweden, Netherlands, Italy, Australia	55	MRD, haplo, third party, BM	II-IV	Early	1.4x10e6, 0.4-9x10e6, 1 (n=27), 2 (n=22), 3-5 (n=6) infusions, dosed weekly	30 CR (55%) 9 PR (16%)	Responders had improved 2-year OS (52 v. 16%, p=0.018) and decreased TRM (13 v. 60%, p=0.002)
2008	Muller *et al.*[63], Germany	2	Haplo, BM	III-IV	Early	0.4-3x10e6, 1 dose	1 CR (50%)	Pediatric study. Both patients had received a haploidentical bone marrow transplant
2008	von Bonin *et al.*[64], Germany	13	Third party, BM	III-IV	Early	0.9x10e6, 0.6x10e6 to 1.1x10e6 ,1-5 doses, dosed weekly	1 CR (8%) 1 PR (8%)	First study of platelet lysate expanded BMSC, disappointing response rate, all but 2 patients required secondary immune suppression

Year published	First author, country	Patient numbers	Donor, BMSC source	GVHD Grade	Passage	BMSC dose (/kg), dose range, number of infusions, number range	Response	Comments
2009	Martin *et al.*[55] (abstract), USA, Canada, Australia	163	Prochymal®, BM	II-IV	Late	2x10e6, 8 infusions, twice weekly, 4 additional infusion if not in CR	ORR 82% BMSC v. 73% placebo (p=0.12)	Prochymal® BMSC performed no better than placebo in this large randomized study. No increase in adverse events compared to steroid alone arm. Better responses in liver/gut. Majority of patients had received second line IS. Results not published.
2010	Lim *et al.*[65], China	1	Third party, BM	III-IV	Early	2x10e6, 2 infusions weeks apart	1 CR (100%)	Explored use of clonal BMSC through subfractionation culture method.
2010	Arima *et al.*[66], Japan	3	MRD, haplo, BM	III	Early	0.5x10e6, 1 intra-arterial infusion	2 PR (66%)	Intra-arterial injection through hepatic artery in liver GVHD not shown to be effective.
2010	Luccini *et al.*[67], Italy	8	Third party, BM	I-IV	Early	1.2x10e6, 1 infusion, with 2-5 infusions given to non-responders	4 CR (50%) 2 PR (25%)	Pediatric study of acute and chronic GVHD. Demonstrates safety of early passage BMSC in pediatric patients.
2011	Prasad *et al.*[68], USA	12	Prochymal®, BM	III-IV	Late	2x10e6 (n=10) or 8x10e6 (n=2) twice weekly for 4 weeks. 4 weekly infusion if not in CR at d+28, median 10 doses	7 CR (58%) 2 PR (17%)	First study of Prochymal® in pediatric patients. Refractory to median 3 other immunosuppressive therapies. No dose response relationship.
2011	Perez-Simon *et al*[69], Spain	10	MRD, haplo, third party, BM	II-IV	Early	1x10e6, 1-4 infusions based on response	1 CR (10%) 6 PR (60%)	Platelet lysate expanded. Refractory to median 3 other immunosuppressive therapies.
2011	Wernicke *et al.*[70], Germany	2	Third party, BM	IV	Early	0.9-1.9x10e6. 1 infusion each	2 CR (100%)	Two pediatric patients, severe life threatening GVHD, multiple concurrent IS therapies.
2011	Wu *et al*[71]. Taiwan	2	Third party, UCB	IV	Early	3.3-4.1x10e6, 1 infusion each	2 CR (100%)	Two pediatric patients, severe life threatening GVHD, multiple concurrent IS therapies. First study using umbilical cord blood.
2011	Herrmann *et al.*[72]. Australia	12	MRD, haplo, third party, BM	I-IV	Early	1.7x10e6, median 2 infusions, 1-19 infusions	7 CR (58%) 4 PR (33%)	6 of 7 patients with CR had long term survival. One hepatic CR after 19 infusions.
2012	Dander *et al.*[73], Italy	6	Third party, BM	II-IV	Early	0.9-1.9x10e6, 2-3 infusions	1 CR (17%) 4 PR (66%)	Showed IL2Ra and TNF-R decreased with BMSC therapy.
2012	Chen *et al.*[74], China	19	Third party, UCB	II-III	Early	0.6-7.2x10e6, 1-3+ infusions	11 CR (58%) 4 PR (21%)	Largest group of patients treated with UCB derived BMSC.
2013	Muroi *et a.l*[75], Japan	14	Third party, Prochymal® collaboration, BM	II-III	Late	2x10e6, 8 infusions, twice weekly, 4 additional infusion if not in CR	8 CR (57%) 5 PR (36%) ORR 92.9% at day +28.	Early administration. BMSC given at progression within 3 days or no response within 5 days of initial corticosteroids, impressive overall response.
2013	Resnick *et al.*[76] Israel	50	MRD, haplo, third party, BM	II-IV	Early	1x10e6, 0.3-4.3x10e6 1-4 infusions	17 CR (34%) ORR 66%	Steroid resistant grade IV GVHD in 84% of patients. Better response and OS seen in GVHD < grade IV, pediatric group. BMSC responders had higher OS. Two cases intra-hepatic artery injection not effective.
2013	Ball *et al.*[77], Sweden, Netherlands, Italy, Canada	37	Third party, BM	III-IV	Early	2x10e6 for median 2 doses, range 1-13	24 CR (65%) 8 PR (22%)	The children treated between 5 and 12 days after steroid initiation (as opposed to later) showed a trend towards better OS (56% v. 25%) and lower TRM (17% v 53%). 12 children received 3-5 doses.
2013	Introna *et al*[78], Italy	40	Third party, BM	II-IV	Early	1.5x10e6, median 3 infusions	CR 11 (27.5%) PR 16 (40%) ORR 67.5 %	Pediatric and adult. Platelet lysate expanded. Less than half of patients receiving secondary IS. Plasma levels of IL2R lower in responders.

Year published	First author, country	Patient numbers	Donor, BMSC source	GVHD Grade	Passage	BMSC dose (/kg), dose range, number of infusions, number range	Response	Comments
							at day +28	
2014	Sanchez-Guijo *et al.*[79],Spain	25	Third party, BM	II-IV	Early	1.1x10e6 day 1, 4, 11, 18, additional doses given for P	11 CR (44%) 6 PR (27%) ORR (71%)	Platelet lysate expanded. Median time to response 28 days (range 9 to 58). Only 33% ORR for liver GVHD
2014	Yin and Battiwalla *et al.* [80], NIH	7	Third party, BM	II-IV	Early	2x10e6, 3 infusions day 0, 7, 14	5 CR (71%)	Showed Reg3a, elafin, CK18, and cytokines decreased in responders.
2014	Kurtzberg *et al.* [81], USA, Canada	75	Prochymal®, BM	II-IV	Late	2x10e6, 8 infusions, twice weekly, 4 additional infusion if not in CR	ORR 61.3 % at day +28	Pediatric study. Starting treatment 88% had grade III (28.0%) or grade IV (60.0%) acute GVHD. 60% of patients had received two or more additional IS therapy. 40 of 75 patients (53.3%) received more than 8 infusions and were included in the continuing therapy analysis. More than one-half of those patients (57.5%) demonstrated additional improvement, with 16 patients achieving complete resolution. Same dosing regimen proposed in T-H-0325.
2014	Zhao et al.[82], China	28	Third party, BM	II-IV	Early	1x10e6 weekly until complete response or 8 total doses	ORR 75% at day CR 17 (61%) PR 4 (14%)	Compared prospectively to a 19 patient control arm. ORR in intervention group 75% and 42.1% in control arm. No increase in CMV/EBV reactivation. Noted increase in Tregs and TRECs.

Table 2 - Summary of reported BMSC use for refractory GVHD

2.5 NIH PHASE I BMSC TRIAL (12-H-0010)

2.5.1 NIH Phase I Clinical Trial Design

Results from the Phase I trial of BMSC at NIH were recently published[39] and reviewed[83]. At the NIH Clinical Center, we developed a clinical grade BMSC cell bank from third party donors[37,38]. We approached the development of BMSC by selecting the EU method of using early passage BMSC. The infusion schedule of doses of $2x10^6$ BMSC/kg weekly followed previously used schedules effective for controlling GVHD[84]. We conducted the phase I trial using our third party, early passage BMSC for patients with steroid-resistant liver or gastrointestinal GVHD, tissue injury or marrow failure following allogeneic stem cell transplant. The study schema is depicted in Figure 1.

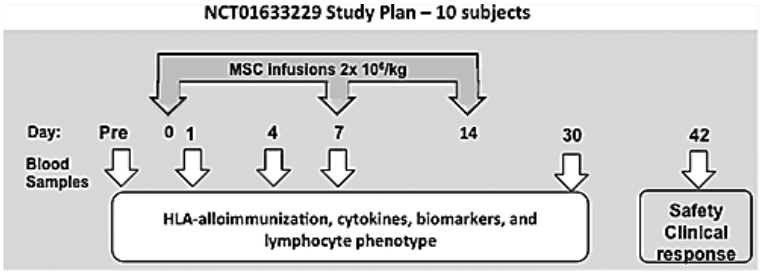

Figure 1 - 12-H-0010 study schema

The BMSC were prepared from marrow aspirates from healthy volunteers with the expansion of 3 passages[38]. Ten subjects were infused a fixed dose of $2e10^6$ BMSC/kg intravenously weekly for three doses. Nine of the subjects had acute GVHD as the primary indication. There was no treatment related toxicity (primary endpoint). In addition to the demonstration of safety and exploration of efficacy, the major objective was to understand the biology of BMSC in post-transplant complications. The infused

BMSC were fully characterized in terms of viability, freeze-thaw characteristics, and gene expression profiles with the goal of identifying markers for potency. We examined the impact of BMSC in terms of their immunogenicity by HLA-alloimmunization, their homing, and fate (by chimerism analysis). Advances in new diagnostic tools using GVHD-relevant biomarkers and markers of tissue injury provided the opportunity to more clearly define responses to BMSC[85]. Patients were monitored for validated plasma GVHD biomarkers, cytokines, growth factors, and lymphocyte phenotype before and after BMSC infusion to identify mechanisms of BMSC immunomodulation and tissue repair.

2.5.2 NIH Phase I Clinical Trial Results

Eight of nine subjects with steroid-resistant acute GVHD were evaluable for response assessment at 4 weeks after the last infusion. There were 5 complete responses, 2 partial responses, and one patient who did not respond. Rapid reductions in markers of tissue destruction and inflammatory cytokines occurred after the first BMSC infusion in several patients, example shown in Figure 2.

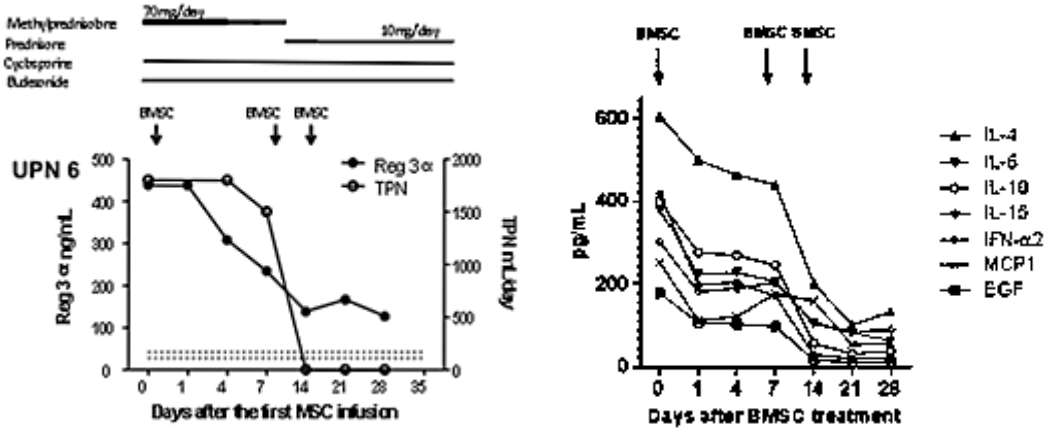

Figure 2 – Patient with a complete response after BMSC treatment (left), and corresponding fall in cytokines (right)

Clinical responses correlated with a fall in biomarkers (Reg 3a, CK18, and Elafin) relevant for the site of GVHD, or CK18 for tissue injury. Response to BMSC therapy and changes in cytokines and GVHD biomarkers segregated with survival.

2.6 Safety Of BMSC Infusions

Safety is a significant concern when translating any new therapeutic into clinical practice. Issues specific to BMSC therapy include the risk of infusion toxicity; an increased susceptibility to infection given their immune-modulatory effects; the dissemination of zoonoses associated with cell culture reagents; an increased neoplastic potential due to the proliferative capacity of BMSC; the potential for embolism of cells; and, the immunogenicity of the cells themselves[86]. BMSC have been used safely in clinical practice for over 10 years; neither acute nor long-term adverse events have been reported after infusion of autologous, donor-derived, or third party BMSC[83]. Safety of intravascular BMSC infusions in humans was reviewed systematically in October, 2012[53]. In a meta-analysis of 36 studies that were deemed eligible, including eight RCTs, the authors were unable to detect an association between BMSC treatment and infusion toxicity, infection, malignancy, acute organ dysfunction, and death. In fact, six of seven RCTs and all non-RCTs described equal or fewer deaths with BMSC treatment compared to control treatment. The only significant association with BMSC treatment was transient fever. Our

chosen dosing schedule of $2x10^6$cells/kg/dose twice weekly for four weeks with additional dosing for partial responders is more treatment than in many earlier trials of early passage BMSC, but this same regimen has been shown to be safe and effective in studies using Prochymal®, the universal donor, late passage BMSC product and another similar BMSC product that is being developed in Japan[75,81].

2.6.1 Safety of the NIH BMSC Product

The phase I study met the primary objective of safety[39]. Protocol defined treatment-related SAE was encountered by none of the subjects. Infusion reactions were seen in one subject (grade 1 pruritus). There was one death while on study (gram-negative bacterial sepsis). There were seven serious adverse events: bacterial infection (n=5), gastrointestinal bleeding (n=1), and a mucus plug in the respiratory tract (n=1). In addition, there were 13 non-SAEs: grade 1 (n=7), grade 2 (n=4), and grade 3 or 4 (n=2). None of these adverse events were attributable to the BMSC product. The HLA antibody screen remained negative prior to and immediately after completing the BMSC trial. New CMV reactivation without the development of CMV organ diseases occurred in two patients, controlled by pre-emptive antiviral therapy.

2.6.2 Safety in Pediatric Patients

Several studies have shown significant clinical benefit of BMSC for refractory acute GVHD in the pediatric setting, which are at least comparable to their adult counterparts, and there have been no reports of serious adverse events attributable to BMSC infusion (see Section 3.8.4). Published pediatric studies with BMSCs with doses as high as $8x10^6$ cells per kilogram body weight have demonstrated safety and efficacy for steroid refractory GVHD and to enhance engraftment[43,63,67,81,87]. A pre-manufactured, universal donor formulation of BMSC (Prochymal®) has been approved as the first stem cell product for refractory acute GVHD in children in Canada and New Zealand[88].

2.7 Scientific And Clinical Justification

The scientific rationale for expanding the use of BMSC to the initial treatment of GVHD is based on preliminary efficacy in the settings of both GVHD prophylaxis and refractory GVHD, the known properties of BMSC to assist in tissue repair and suppress alloimmune responses causing GVHD, and a track record of safety in human subjects. Importantly, treatment with BMSC offers an opportunity to study changes in GVHD biomarkers in the blood before and after BMSC infusion and before, during, and after the onset of GVHD. One of the major obstacles in GVHD research is that GVHD is a heterogeneous illness and responses to therapy can vary widely from study to study and BMSC preparation to BMSC preparation. One of the keys to understanding responses in GVHD may be the discovery of ST2 as a predictive biomarker of GVHD severity and subsequent mortality related to GVHD[89]. Plasma collected at regular intervals after BMSC infusion will be used to study changes in these cytokines. It has been shown recently that BMSC recipients have increased serum CD73-expressing BMSC exosomes in the blood (submitted for publication), and BMSC exosomes are immunologically active[90].

2.8 Rationale for Changes from Phase I Study

The previous phase 1 study used a dosing schedule of $2x10e^6$ BMSC/kg once weekly for three weeks in refractory GVHD, whereas the proposed pilot study will use a dosing schedule of $2x10e^6$ BMSC/kg twice weekly for four weeks for early GVHD, followed by once weekly for 4 weeks in patients who have a suboptimal response. Below is justification for the change in schedule.

2.8.1 Upfront treatment

Acute GVHD is caused by a cascade of events that ultimately lead to alloreactivity and tissue damage. Mechanistically, reversing the processes underlying GVHD early may break the cycle of alternating tissue damage and immune activation, thereby preventing progression to refractory GVHD. In a pediatric study by Ball *et al.*[77], of 37 children, those treated with BMSC between 5 and 12 days after steroid initiation showed a trend towards better OS (56%) and lower TRM (17%) as compared with patients receiving BMSC 13–85 days after initiation of steroids (25% and 53%, respectively; P = 0.22 and 0.06, respectively). More recent studies have prioritized early treatment with BMSC in refractory GVHD[75,79,81] (see Table 2).

2.8.2 Increased number and frequency of doses

Also in the study by Ball et al cited above[77], the authors retrospectively analyze their results in a series of 37 children with steroid-resistant acute GVHD. Multiple doses of BMSC were associated with a higher rate of CR. Cumulative incidence of transplantation-related mortality (TRM) in patients who did or did not achieve CR was 17% and 69%, respectively. In a more recent manuscript describing the Spanish experience, investigators examined a dosing schedule of $1x10^6$ BMSC/kg on days 1, 4, 11, and 18 for treatment refractory acute GVHD and found an ORR of 71%. Most patients received 3 or more infusions, and additional infusions were allowed in patients achieving a partial response. A maximum of 8 infusions were given in two patients and proved to be safe[79]. In a larger study[81], Kurtzberg *et al.* reported on 75 children with treatment-resistant grade II - IV acute GVHD who received the pre-manufactured, universal donor formulation of BMSC (Prochymal®). The BMSC dose was $2x10^6$ cells/kg/dose infused twice weekly for 4 weeks. In the case of a partial or mixed response, patients were treated with $2x10^6$ cells/kg/dose weekly for four more weeks. This is the same schedule proposed in our study. Patients received a median of 10 doses. At the start of treatment, the majority of patients had grade III (28.0%) or grade IV (60.0%) refractory acute GVHD and patients were heavily pretreated with immunosuppression. The overall response rate was 61.3% at day 28. No infusion or other identifiable acute toxicity was seen in any patient.

2.8.3 Continuation of therapy

In the pediatric study previously cited by Kurtzberg *et al.*[81], in the case of a partial or mixed response after $2x10^6$ cells/kg/dose twice weekly for 4 weeks, patients were treated with $2x10^6$ cells/kg/dose weekly for an additional four weeks and assessed again for response. For a patient to be considered a responder between day 28 and day 100, they must have experienced additional improvement in at least 1 organ of at least 1 stage without worsening in any other organ. Forty (40) patients on this study received more than the planned 8 infusions and more than one-half of these patients (57.5%) demonstrated additional improvement in acute GVHD, with 16 patients achieving complete resolution of acute GVHD.

2.8.4 Addition of pediatric patients

Several studies have shown significant clinical benefit of BMSC for acute GVHD in the pediatric setting, which are at least comparable to their adult counterparts. In the largest cohort of children to date[68,81], Kurtzberg *et al.* reported on 75 children with treatment-resistant grade II - IV acute GVHD who received Prochymal®. The BMSC dose was $2x10^6$ cells/kg/dose, and infused twice weekly for 4 weeks. In the case of a partial or mixed response, patients were treated with $2x10^6$ cells/kg/dose weekly for four more weeks. Patients received a median of 10 doses. At the start of treatment, the majority of

patients (88%) had grade III (28.0%) or grade IV (60.0%) acute GVHD. Many patients (60%) had received two or more immunosuppressive therapies in addition to steroids. The overall response rate was 61.3% at day 28. No infusion or other identifiable acute toxicity was seen in any patient. Prochymal® is approved for use in Canada and New Zealand for refractory GVHD in pediatric patients. Similar results have recently been reported in a smaller European pediatric study of eleven (11) patients with acute or chronic GVHD (grade I to IV) who received HLA-disparate donor's bone marrow-derived BMSC, expanded in platelet-lysate containing medium on compassionate use studies[67]. The median dose administered to patients (aged 4-15 years) was 1.2 x 10^6 cells/kg/dose (range: 0.7-3.7x10^6 cells/kg/dose). No acute or late side effects were reported with a median follow up of 8 months. The overall response rate was 71.4% (CR = 23.8%). Müller *et al.* reported on the administration of ex vivo-expanded BMSC in seven pediatric patients in escalating doses (0.4x 10^6 to 3.0x10^6 cells/kg/dose)[63]. No adverse effects were observed in a follow up of 29 months.

3. STUDY DESIGN

3.1 STUDY DESIGN

This is a pilot trial designed to explore the feasibility of using BMSC combined with standard therapy for the initial treatment of acute GVHD requiring systemic therapy

3.1.1 Accrual Objective

Twenty-five (25) patients will be accrued with the goal of attaining twenty-three (23) evaluable patients.

3.1.2 Accrual Period

The estimated accrual period is 18 months.

3.1.3 Study Duration

The expected maximum duration of treatment according to the study protocol will be 11 weeks total as outlined in Section 3.4.

3.1.4 Rationale for Design

Twenty five patients over 12-18 months is a realistic target for accrual given the incidence of acute GVHD in the clinical center.

3.2 SUBJECT INCLUSION

Provided subjects satisfy inclusion criteria in Section 5.1, the protocol is open to all subjects transplanted at NIH who are diagnosed with acute GVHD, irrespective of conditioning regimen, primary disease, or transplant type. The protocol will include subjects at any time after allogeneic stem cell transplantation.

3.3 INITIAL ASSESSMENT

Subjects will be assessed by clinical laboratory criteria and also where appropriate, by biopsy for degree and severity of GVHD of the liver and/or gastrointestinal tract according to criteria in Appendix 1. Patients with confirmed acute GVHD will be enrolled within 120 hours of receiving systemic corticosteroids for the treatment of acute GVHD.

3.4 TREATMENT

3.4.1 Schema and Treatment Summary

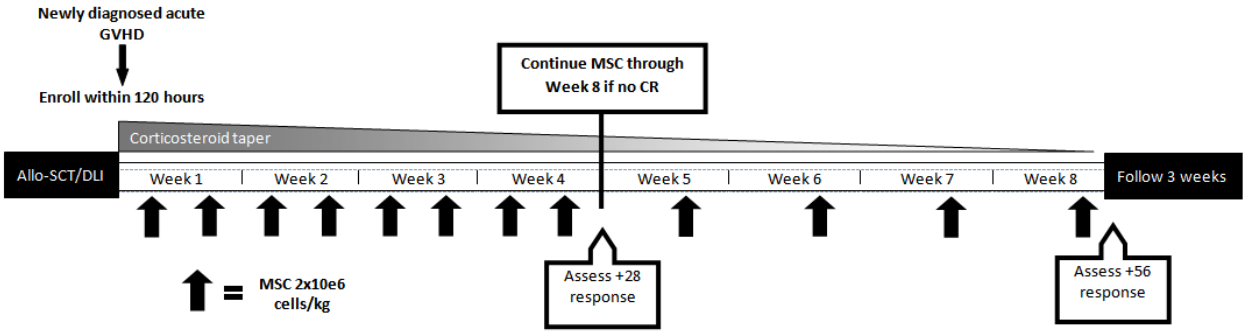

Figure 3 - Study schema

This is a pilot trial where eligible subjects may be entered into this study if they meet the diagnostic criteria for acute GVHD requiring systemic corticosteroid therapy. Subjects will receive the first infusion of BMSC within 120 hours of the first dose of systemic corticosteroids. Each subject will receive $2x10^6$ cells/kg of BMSC given twice weekly, no sooner than 48 hours apart, for 8 doses. Four (4) additional weekly doses will be given in the absence of a complete response. BMSC will be given for a maximum of 12 doses. We will endeavor to administer BMSC on Tuesdays and Fridays in order to facilitate planning with CPS DTM. Each BMSC infusion will contain a target dose of $2x10^6$ BMSC/kg (+/- 10%) viable BMSC (30-150 mL). Donor sample identifiers will be tracked for each infusion. Since there are no specific or antidotal therapies for AEs arising from BMSC, any toxicity that arises during the subject's infusion or post infusion assessments will be managed with standard supportive care as outlined below. Patients will also be treated with systemic corticosteroids per established treatment guidelines (Section 3.4.3).

3.4.2 Treatment with BMSCs

3.4.2.1 Pre-infusion

Pre-infusion steps will be followed according to the NIH institutional standard operating procedure: Administration of Products of Cellular Therapy. Emergency equipment will be made available in the patient room including: oxygen, suction machine, vital signs monitor, and 0.9% sodium chloride solution and administration. All adult subjects will receive diphenhydramine 25-50 mg IV or PO over 10-15 minutes and/or acetaminophen 325-650 mg PO 30-60 minutes prior to infusion; all pediatric subjects will receive diphenhydramine 0.5-1 mg/kg IV or PO over 10-15 minutes (max dose 50 mg) and/or acetaminophen 10-15 mg/kg PO (max dose 650 mg) 30-60 minutes prior to infusion. Subjects will remain in the NIH Clinical Center (inpatient ward or day hospital) to be evaluated for toxicity as specified by the standard operating procedure. Pre-medications may be substituted. Acetaminophen can be held for patients with abnormal liver function studies.

3.4.2.2 Infusion

BMSC infusions will be scheduled with the CPS DTM in advance. BMSC will be thawed rapidly in a 37˚C water bath. BMSC bags will be hand carried by CPS personnel to the patient care unit. BMSC will be administered according to NIH institutional standard operating procedure: Administration of Products of Cellular Therapy. In brief, subjects will be present either in the inpatient ward or the day hospital during the entire time of the infusion. It is recommended to use a central venous catheter for infusion of BMSC, but a patent peripheral access is acceptable. Infusion rate shall not exceed 4-5 mL/min (approximately 10-30 minutes) by gravity. The product should not be given by an infusion

pump. The total dose of the product given with each infusion is $2x10^6$ BMSC/ kg (+/- 10%) viable BMSC (30-150 mL).

3.4.2.3 Post-infusion

Patients will be monitored post-infusion according to the NIH institutional standard operating procedure: Administration of Products of Cellular Therapy. Outpatients may be released thirty minutes to one hour after completion of infusion if patient condition and vital signs are stable, as specified in the standard operating procedure above.

3.4.2.4 Missed infusions

Unanticipated logistical challenges including, but not limited to, adverse weather, availability of transportation, or equipment-related delays may occur. Subjects can miss up to four (4) BMSC infusions in total over the course of the treatment protocol and remain enrolled and evaluable for response. If an infusion or series of infusions are missed, the infusion will not be rescheduled and the subject will go on to receive the next scheduled infusion in the treatment course. Subjects missing more than four (>4) BMSC infusions will be removed from the treatment protocol and will not be evaluable for the primary endpoint.

3.4.3 Treatment with corticosteroids

The optimal dose of steroid and duration of therapy for GVHD is unknown and there is considerable variation in practice, particularly in early stage GVHD, but in general steroid dose should be sufficient to treat GVHD but limited to avoid any side effects of long-term high dose therapy. The preferred rate to taper steroids is also not well-established[91], but general guidelines have been provided in the design of a recent randomized, prospective, multi-center trial (BMT CTN 0802) comparing steroids/placebo vs. steroids/MMF for acute GVHD[6,17]. It is suggested that patients receive an initial steroid dose by IV route (usually methylprednisolone) that is equivalent to prednisone at a dose 2 mg/kg/day at diagnosis of acute GVHD. Steroids can be tapered as tolerated according to individual practice. It is suggested that steroids are tapered if signs of clinical improvement defined as any clinically recognizable lessening of skin rash, redness, or extent; lessening of diarrhea or lowered bilirubin (though it does not have to be greater than or equal to one stage improvement in any involved organ), without worsening in any organ.

	Suggested steroid taper for responders (round to nearest 5mg of prednisone)
Day 1-5	Prednisone 2 mg/kg/day (use IV methylprednisolone equivalent in single or divided dose(s))
Day 6-10	Prednisone 1.5 mg/kg/day PO (or IV methylprednisolone equivalent)
Day 11-15	Prednisone 1 mg/kg/day PO (or IV methylprednisolone equivalent)
Day 16-20	Prednisone 0.5 mg/kg/day PO (or IV methylprednisolone equivalent)
Day 21-28	Prednisone 0.25 mg/kg/day (or equivalent) PO (or IV methylprednisolone equivalent)
Day >28	Continue taper with a goal of <0.2 mg/kg/day of prednisone PO once daily (or equivalent).

Table 3 – Suggested steroid taper

3.4.4 Additional immunosuppressive therapy

3.4.4.1 Standard GVHD prophylaxis

Most patients will receive medications that are considered GVHD prophylaxis as part of their transplant regimen. Patients developing acute GVHD during GVHD prophylaxis therapy (e.g. calcineurin inhibitor, sirolimus, MMF, etc.) should have this medication continued during the study if possible. Patients who have been tapered off of or completed standard calcineurin inhibitor based prophylaxis (e.g. cyclosporine, tacrolimus) can be restarted on this medication concurrent to treatment on this

protocol. Concurrent or addition of topical steroid therapy (skin creams, oral budesonide, or other locally acting steroid preparation) is allowed.

3.4.4.2 Progression of GVHD

If there is no response to therapy or acute GVHD progresses, then the patient can be treated by adding one of many alternative systemic secondary GVHD therapies. BMSC therapy will continue per protocol design, given the prior established efficacy of BMSC in refractory GVHD and the poor outcomes associated with primary refractory and progressive acute GVHD.

3.4.4.3 Flare of GVHD

If acute GVHD flares during taper of steroids or GVHD prophylaxis medications, steroid or GVHD prophylaxis dosing may be re-escalated or secondary therapy added at the discretion of the treating physician. Re-escalation of steroid or GVHD prophylaxis medications for GVHD flare alone will not be considered as need for secondary therapy.

3.4.5 Retreatment with BMSC

There will be no retreatment with BMSC infusions after BMSC treatments are stopped.

3.4.6 Concurrent Treatments

Subjects may receive any concurrent treatment except other investigational agents for treatment of acute GVHD (See PBSCT Supportive Care Guidelines**)**

3.5 TOXICITY

BMSC infusion toxicity will be defined as alteration in vital organ function within the 11 weeks of the study that cannot be explained by other complications; for example, post-stem cell transplant, infections, underlying transplant indication, or other therapy. See Section 8 for definition of Treatment Limiting Toxicity.

3.6 RESPONSE

Subjects will be assessed for response for the primary indication according to criteria listed in Appendix 2. The critical time points for response are at day +28 and day +56. Subjects are considered off protocol after 11 weeks of total enrollment. Subjects will receive additional infusions after day +28 based upon whether or not there is a complete response at the day +28 response assessment (Section 3.4.1). After coming off protocol, subjects will be followed for best response for up to 1 year on their parent transplant protocols.

3.7 SURVIVAL

Survival assessment will be done at day +28 and day +56. After coming off protocol, subjects will be followed for survival for up to 1 year on their parent transplant protocols.

4. RECRUITMENT AND REGISTRATION

The study will be listed on clinicaltrials.gov, clinical center research studies, PDQ, Aplastic Anemia Foundation, and the NHLBI patient recruitment websites. If recruitment goals are not met, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment to extend the protocol to subjects outside NIH.

5. ELIGIBILITY ASSESSMENT

5.1 INCLUSION CRITERIA

5.1.1 History of any grade acute GVHD requiring systemic therapy after allogeneic stem cell transplant or DLI.

Subjects must have received an allogeneic stem cell transplant at NIH and be diagnosed with acute GVHD. Acute GVHD is defined using the NIH consensus definition inclusive of classic acute ($\leq$ 100 days after transplant or DLI, presence of acute GVHD features, absence of chronic GVHD features) AND persistent/recurrent/late onset acute (>100 days after transplant or DLI, presence of acute GVHD features, absence of chronic GVHD features). Subjects with stage I and II skin only (overall Grade I) or isolated upper gastrointestinal involvement are eligible if the treating physician deems that systemic corticosteroid treatment is indicated. Biopsy confirmation of GVHD is desirable, but not required for study entry because enrollment should not be delayed awaiting biopsy or pathology results. Patients must be diagnosed with a first episode of acute GVHD requiring systemic corticosteroids and associated with preceding administration of a cellular therapy including stem cells and donor lymphocyte infusion. Patients who were treated for GVHD associated with another cellular therapy product (e.g. prior allogeneic transplant or DLI) will be allowed into the study.

5.1.2 Previous immunosuppressive therapy

The patient must have received no systemic immune suppressive therapy for treatment of new acute GVHD (e.g. pentostatin, etanercept, denileukin difitox, etc.), except for a maximum 120 hours prior corticosteroid therapy. This does not include immune suppressive therapy for GVHD prophylaxis (e.g. calcineurin inhibitor, sirolimus, MMF, etc.). It is expected that most patients will be receiving GVHD prophylaxis as part of their transplant regimen, thus patients developing acute GVHD while on GVHD prophylaxis will still be considered eligible. Concurrent or addition of locally-acting steroid therapy (skin creams, oral budesonide, or any other locally-acting steroid preparation) is allowed.

There is one exception to the above stipulations: Use of the oral medication MMF (in addition to systemic corticosteroids) for the treatment of acute GVHD will be allowed. MMF is commonly given early in the treatment of acute GVHD, but it has not been shown to improve outcomes compared to steroids alone in a randomized, prospective study; therefore, treatment with MMF will not exclude patients from BMSC treatment.

5.1.3 Age

Age $\geq$ 4 years old will be allowed.

5.1.4 Birth control

Subjects of childbearing or child-fathering potential must be willing to use a medically acceptable form of birth control, which includes abstinence, while they are being treated on this study.

5.1.5 Informed consent

Signed informed consent and/or assent is required. Assent and educational materials will be provided to, and reviewed with, patients under the age of 18. The informed consent process will begin at recognition of patient eligibility.

5.2 Exclusion Criteria

5.2.1 Breast feeding or pregnant females (due to unknown risk to fetus or newborn).

5.2.2 Known allergy to gentamicin.

6. CLINICAL EVALUATION

6.1 CLINICAL EVALUATION OF THE SUBJECT

6.1.1 Eligibility Evaluation

a) Karnofsky performance status

b) Eligibility to participate in trial

c) Pregnancy test in females of child-bearing potential

d) Relevant GVHD organ-specific studies, confirmation of diagnosis, and severity assessment (Appendix 1)

e) Acute GVHD diagnosed by clinical criteria and no more than 120 hours of prior steroid therapy for diagnosis

Note that eligibility screening may be performed on a separate NIH-CC screening protocol

6.1.2 Pre-Treatment Evaluation

Pretreatment evaluation is to be completed within 30 days of the protocol enrollment date.

a) Physical examination

b) Vital signs: temperature, pulse oximetry, blood pressure, respiratory rate, height and weight

c) CBC with differential + reticulocyte

d) Chemistry panel (Na, K, Cl, CO2, Cr, glucose, BUN, albumin, Ca, Mg, Phosphorus, Alk Phos, ALT/AST, total and direct bilirubin, LDH, total protein, CK, uric acid)

e) As per GVHD evaluation in Appendix 1

6.1.3 Toxicity monitoring

a) Administration and monitoring of BMSC will be performed according to NIH Clinical Center standard operating procedure: Administration of Products of Cellular Therapy.

6.1.4 Follow up Evaluations

a) Physical exam and performance score

b) Vital Signs

c) Routine CBC with differential, Chemistry panel (Na, K, Cl, CO2, Cr, glucose, BUN, albumin, Ca, Mg, Phosphorus, Alk Phos, ALT/AST, total and direct bilirubin, LD, total protein, CK, uric acid), and reticulocyte count.

d) Acute GVHD severity and response monitored by skin exam, stool volume, and liver function tests on days of BMSC infusion by the investigators while inpatient and during the day of clinic visit as outpatient.

Follow up evaluations after the last BMSC infusion will be based on subject's condition, and may be as frequent as twice weekly. All radiographic examinations at baseline and follow up would be considered standard of care for the evaluation and management of these protocol subjects as clinically warranted.

6.2 Response Definitions

6.2.1 Response

Response criteria are defined in Appendix 2. Response will be recorded at day +28 and day +56. Best response will also be recorded: i.e. the maximum improvement achieved following BMSC infusion in the follow up period between day +56 and one year post transplantation.

7. SAMPLE COLLECTION, STORAGE AND TRACKING PLAN

7.1 Research Sample Collection

All research sampling will be obtained based upon the clinical situation and may be optional at the discretion of the PI. Such situations may occur without violating the protocol when the PI determines that sample collection is precluded by the subject's performance status, or would interfere with management of an acute medical situation or when logistic considerations prevent procurement or processing of the specimen.

7.1.1 Research Blood Sampling:

Research blood samples: 54 mL of blood will be drawn [1 (6 ml) lavender top- K2-EDTA for plasma and 6 (8 ml) red-green "tiger top"] on Day 0 (pre BMSC infusion), Day 28, and Day 56. 3 mL of blood [1 (3 ml) lavender top- K2-EDTA for plasma] will be drawn before and after each BMSC infusion (+/- 24 hours) and at 3 weeks after the last infusion (+/- 7 days). Testing will include for cytokines, GVHD biomarkers, plasma exosomes, lymphocyte subset analysis and other Stem Cell Allotransplantation Section research studies (Appendix 3). For logistic considerations, a +/- 3-day window can be applied to sample draw dates on D0, D28, and D56. Blood will be stored at room temperature and transported to the laboratory of Dr. John Barrett, NHLBI, NIH for analysis. A list of IRB-approved research tests that will be conducted on the collected samples is included in Appendix 4.

Pharmacokinetics (PKs): In selected patients, and after selected infusions (as determined by the investigator, clinical course, and available nursing/lab resources), up to 10 blood samples will be drawn for PKs to determine the kinetics of BMSC-derived exosome release. 2mL of blood in 3mL lavender top- K2-EDTA tubes will be drawn at intervals following infusion. The suggested intervals are 20 min, 40 min, 1 hr, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 12 hrs, 18 hrs, and 24 hrs from infusion. The suggested intervals are not mandatory and can be modified as needed based on nursing/laboratory availability and clinical course.

7.2 Collection, Storage And Disposition Of Samples

7.2.1 Intended use

Any research specimen will not be read by a pathologist or used for diagnostic purposes. These studies will not be used in assessing clinical endpoints but are undertaken for descriptive or exploratory ancillary research.

7.2.2 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. Samples will not be sent outside NIH without IRB notification and an executed MTA.

7.2.3 Storage

Research samples will be stored with identifiers in the secure laboratory of Dr. John Barrett, NHLBI, NIH. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI).

7.2.4 End of study procedures

The study will remain open so long as sample or data analysis continues to a maximum of one year after the last subject completes treatment. 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.

7.2.5 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.

8. MANAGEMENT OF COMPLICATIONS

8.1 OVERVIEW

Serious toxicities related to infusion of BMSC have not been reported. Hives and nasal congestion have been identified infrequently. Infusion associated fever is recognized. As with any infusion of a cell product, immediate transfusion reactions are a theoretical possibility.

8.2 IMMEDIATE TRANSFUSION REACTION

Administration of BMSC will be performed according to NIH Clinical Center standard operating procedure: Administration of Products of Cellular Therapy. If an allergic or other acute reaction occurs, studies appropriate for investigation of a transfusion reaction will be performed (urinalysis, CBC, Coomb's test). Any adverse reactions should prompt interruption of the infusion and should be reported to the principal investigator.

No further BMSC infusions will be administered to a subject in the event of:

- recurrent infusion related toxicity *despite premedication* (moderate toxicity is defined as requiring interruption or discontinuation of BMSC administration, which is not rapidly responsive to symptomatic medication)
- any CTCAE v4.0 grade 4 acute infusion reaction (defined as requiring discontinuation of infusion AND urgent cardiopulmonary intervention) or grade 4 hypersensitivity reaction (anaphylaxis) at least possibly related to BMSC infusions
- any CTCAE v4.0 grade 3 infusion reaction that does not resolve completely within 2 hours
- any CTCAE v4.0 grade 3 acute infusion reaction or hypersensitivity that recurs when the infusion is restarted.

8.3 TREATMENT LIMITING TOXICITIES

The observational period for determining Treatment Limiting Toxicities is from the initiation of the BMSC infusion to 3 weeks after the last infusion. A treatment related serious adverse event (TRSAE) is defined as an SAE which is at least probably or definitely related to the BMSC infusions. Any subject who encounters a TRSAE will not receive further infusions.

8.4 POST-TRANSPLANT COMPLICATIONS

New post-transplant complications will be managed according to standard NIH Intramural transplant guidelines (See PBSCT Supportive Care Guidelines).

9. OFF TREATMENT AND OFF STUDY CRITERIA

9.1 OFF TREATMENT CRITERIA

Subjects will receive no further BMSC infusions if any of the following occur:

9.1.1 Toxicity

Subjects experiencing BMSC infusion reaction as defined in Section 8.2 will receive no further BMSC infusions; subjects experiencing a grade 4 acute infusion reaction or hypersensitivity reaction will receive no further treatments; subjects experiencing a grade 3 acute infusion reaction or hypersensitivity reaction will receive no further treatments if symptoms do not resolve within 2 hours; subjects experiencing a grade 3 acute infusion reaction or hypersensitivity reaction will receive no further treatments if symptoms recur when infusion is restarted. Any subject who encounters a TRSAE will not receive further infusions. Subjects will be followed until off study criteria are met.

9.1.2 Completion of Infusions

A maximum of 12 infusions is planned. Final evaluation of outcomes and response to the BMSC will occur 3 weeks after the last infusion. After planned infusions and the three week monitoring period, subjects will be taken off protocol. Subjects will be followed for GVHD response and survival as a secondary endpoint for up to 1 year on their parent transplant protocol.

9.1.3 Subject Choice

The subjects are at liberty to withdraw from further infusions at any time.

9.2 OFF STUDY CRITERIA

9.2.1 Withdrawal per subject choice

Subjects are at liberty to withdraw from the protocol at any time. They will continue to receive standard care for their post-transplant complication.

9.2.2 Subject Death

9.2.3 Withdrawal by physician decision

Subjects may be withdrawn from the protocol at any time if the PI deems that the subject will not survive to be evaluable beyond 7 days from transfusion, or that BMSC infusion will complicate the routine management of the recipient. In addition, subjects who are noncompliant with treatment or

monitoring and those who develop such severe inter-current illness that compromises treatment or evaluation will be taken off study.

9.2.4 Completion of the study

At the conclusion of this study, all surviving subjects will be taken off study or if appropriate enrolled on a follow up study to obtain survival data.

10. DATA MANAGEMENT

10.1 DATA SAFETY

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators, Hematology Branch fellows, research nurses and/or a contracted data manager will assist with the data management efforts. Some primary source data on baseline characteristics, BMSC infusions, adverse events, concomitant medications and clinical response will be captured directly on case report forms (CRFs). Data will also be abstracted from Clinical Center progress notes. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database.

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. 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) or other unique code, or minimum PII required for subject identification. All protocol data will be stored in the NHLBI secured network drive (P drive) with the following security measures: restricted access, password protection and daily off-site back-up.

10.1.1 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.

10.1.2 Loss or destruction of data

Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

10.1.3 Future use of data

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 IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP). No identifiable data will be sent outside NIH without IRB notification and an executed MTA or CTA.

11. BIOSTATISTICAL CONSIDERATIONS

11.1 PRIMARY ENDPOINT

The primary endpoint of this study is:

- The proportion of subjects reaching day +56 without a TRSAE.

11.2 Secondary Endpoints

Secondary endpoints for this study are:

- Proportions of complete response (CR), partial response (PR), mixed response (MR), no response (NR), and worsening disease (WD) among surviving patients at day +28 and +56 of study (Appendix 2). Need for secondary immunosuppression by day +28 and +56 of study. Steroid dose (prednisone equivalent) at day +28 and +56 of study. Proportion of responses in patients with high and low ST2 levels.

Secondary endpoints will not be used in assessing success or failure of the primary endpoint but are undertaken for descriptive or exploratory research and will be reported accordingly. The duration of treatment limiting toxicity monitoring will be 3 weeks after the last dose of BMSC. The total duration of treatment limiting toxicity monitoring will be 7-11 weeks. Total serious adverse event monitoring will continue until at least 3 weeks after the last dose of BMSC, and subjects experiencing adverse events related to BMSC will be monitored until toxicity resolution or stabilization.

11.3 STATISTICAL ANALYSIS

11.3.1 Methods of Statistical Analysis

The planned analyses will include descriptive statistics on response rates, biomarkers, and the incidence and severity of adverse events. The proportions of treatment related adverse events and their severity will be estimated using the sample proportions, and their inferences including confidence intervals and hypotheses testing will be evaluated using Binomial distributions. The time to adverse events will be analyzed using appropriate tools in survival analysis such as Kaplan-Meier estimates. Graphical tools will be used to display the appropriate estimates.

11.3.2 Sample Size

Let p be the probability of subjects reaching day +56 without a TRSAE. The sample size is determined by testing the null hypothesis H0 : $p = 75\%$ versus the alternative hypothesis Ha: $p > 75\%$ with a one-sided significant level of 0.05. Based upon our previous phase 1 trial (12-H-0010), the TRSAE rate was 0 out of 10 subjects and we expect 95% subjects would reach day +56 without a TRSAE. Thus a sample size of 23 subjects will achieve 89.5% power to test the above hypotheses using an exact binomial test.

To account for the possibility of non-treatment related drop out, up to 2 additional subjects may be enrolled to replace those subjects that are non-evaluable for the study's primary endpoint for a total accrual of 25 patients. At an accrual rate of 1-2 subjects per month, this study will be completed in 12-18 months.

11.3.3 Subject Replacement

Subjects who do not complete at least two doses of BMSCs will be replaced in this study.

11.3.4 Stopping Rules

11.3.4.1 For Mortality

Any death occurring through the follow up period (3 weeks after last BMSC infusion) will halt further accrual irrespective of causality pending IRB review. The study will continue only with IRB determination that the event was not related to study procedures.

11.3.4.2 For Safety

The trial will be monitored for safety based on Treatment Related Serious Adverse Events (TRSAE), which is also the primary endpoint of the study. A TRSAE is defined as an SAE which is at least probably or definitely related to the BMSC infusions. The trial will be stopped if 3 subjects have developed one or more TRSAE's. This is based on the binomial test of the primary hypothesis (Section 11.3.2) and at least 21 of 23 subjects without a TRSAE are needed to reject the null hypothesis. If 3 or more subjects developed TRSAE's are observed, the null hypothesis will not be rejected and we will stop the study early.

12. DATA AND SAFETY MONITORING

12.1 SAFETY MONITORING

12.1.1 Principal Investigator

Accrual and safety data and conduct of the trial will be monitored by the PI and research team on an ongoing basis. The protocol will be continuously evaluated for any unusual or unpredicted complications with the aim of detecting and preventing unacceptable increase in morbidity and mortality over and above that anticipated from standard allogeneic stem cell transplants. (See stopping rules in section 11.3.4). Given that safety was established in the prior Phase 1 study, non-serious adverse events will not be collected (see definition Section 12.2).

12.1.2 NHLBI IRB

Prior to implementation of this study, the protocol and the proposed subject consent and assent forms will be reviewed and approved by the properly constituted IRB operating according to the 45 CFR 46, 21 CFR 50 and 56. This committee must approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or sample and/or data analysis continues.

12.1.3 DSMB

The NHLBI Data Safety and Monitoring Board will review the protocol annually or semiannually. A progress report will be forwarded to the DSMB at this time. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

12.1.4 FDA

An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to FDA.

12.1.5 Data Integrity and Study Conduct

Regularly scheduled clinical trial monitoring will be performed by experienced contracted clinical trial monitors according to established procedures established by the Office of Clinical Affairs, NHLBI.

12.2 ADVERSE EVENTS

12.2.1 Definitions

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

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Serious Adverse Event (SAE): A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

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

Serious event: An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

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

Unanticipated Problem (UP): Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

1. **unexpected** in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. **related or possibly related** to participation in the research; and
3. places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Non Compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. Serious non-compliance: Non-compliance that:
   a. Increases risks, or causes harm, to participants.
   b. Decreases potential benefits to participants.
   c. Compromises the integrity of the NIH HRPP.
   d. Invalidates the study data.

2. Continuing non-compliance: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. Minor (non-serious) non-compliance: Non-compliance that, is neither serious nor continuing.

12.2.2 Adverse Event Management

The following adverse event management guidelines are intended to ensure the safety of each subject while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution

Relationship	**Attribution**	**Description**
Unrelated to investigational agent/intervention[1]	Unrelated	The AE *is clearly* ***NOT*** *related* to the intervention
	Unlikely	The AE *is* ***doubtfully related*** to the intervention
Related to investigational agent/intervention[1]	Possibly	The AE ***may be related*** to the intervention
	Probably	The AE ***is likely related*** to the intervention
	Definitely	The AE ***is clearly related*** to the intervention

[1]**NOTE**: AEs listed as 'possibly, probably, or definitely' related to the investigational agent/intervention are considered to have a suspected 'reasonable causal relationship' to the investigational agent/intervention (ICH E2A).

The following are expected outcomes for the transplant recipient (not necessarily as a result of this study) and will be documented in the subject's medical record but not reported to IRB unless they meet the criteria for an SAE or UP:

- Renal insufficiency
- Hepatic insufficiency
- Transient cardiac arrhythmias
- Transient cardiac insufficiency
- Pulmonary insufficiency
- Neutropenia and its complications
- Thrombocytopenia and its complications
- Anemia and its complications
- Transfusion reactions
- Treatable infections from bacteria, viruses, protozoa and fungi
- Late effects of transplant regimens including: chronic fatigue, cataracts, infertility, growth impairment, hypothyroidism, bone complications, and dental caries
- Headache, insomnia, psychosis, mood changes, disorientation, seizures from metabolic imbalance
- Nausea, vomiting, diarrhea, mucositis, weight loss, dry mouth, hiccoughs, constipation
- Well-characterized drug reactions - allergic manifestations, "red man" syndrome, steroid effects
- Well-characterized drug side effects from drugs used routinely in transplant recipients (e.g.; preparative regimen chemotherapy, immunosuppressive drugs, antimicrobials)
- Common side effects of antiemetics, analgesics, anti-inflammatory agent and known complications of steroid therapy

- Complications from intravenous catheters, thrombotic occlusion, infection, local reactions, cardiac arrhythmia
- Expected adverse events related to investigational reagents and transplant drugs are listed in section 13.3.

The following are expected transplant outcomes that will be reported in summary form at the time of continuing review but will not be reported to IRB at each occurrence unless they meet the criteria of an SAE or UP:

- Acute graft-versus-host disease
- Chronic graft-versus-host disease
- Graft failure / graft rejection
- Veno-occlusive disease
- Hemorrhagic cystitis
- Cytomegalovirus reactivation or disease
- Autoimmune phenomena
- EBV reactivation or disease
- Fungal infections
- Disease relapse or progression

12.3 NHLBI IRB AND CD REPORTING

12.3.1 Serious Events

Reports to the IRB and CD:

The PI must report Serious UPs and Serious PDs, to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event via iRIS using the NIH Problem Form.

Reports to the IRB Chair and CD

The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event via iRIS, using the NIH Problem form.

12.3.2. Non-serious Events

Reports to the IRB and CD:

The PI must report all UPs that are not Serious to the IRB and CD, and PDs that are not Serious to the IRB, not more than 14 days after the PI first learns of the event via iRIS using the NIH Problem Form.

12.3.3 Deaths

The PI must report all deaths (that are not UPs) to the CD as soon as possible, but not more than 7 days after the PI first learns of the event.

12.4 IND SPONSOR REPORTING CRITERIA

An investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Events will be submitted to Dr. A John Barrett, IND Sponsor at:

A. John Barrett, M.D.

Rm 3-5330, HB, NHLBI, CRC,

10 CenterDrive,

Bethesda, MD 20892-2012.

12.5 DSMB Reporting

Any UP observed during the clinical trial and for which there is a relationship with the use of BMSC cells and the conduct of the study will be reported within 24 hours to the Data and Safety Monitoring Board (DSMB). A summary of all SAEs and AEs will be included for review at the regularly scheduled DSMB meeting.

12.5.1 Serious Adverse Event Reporting on Cell Therapy Products to the NHLBI IRB and FDA

If a cell product deviation (or any component thereof) occurs during or after its manufacture, a report of the event, and information relevant to the event, associated with the manufacturing will be submitted to the NHLBI IRB and the FDA by the PI and/or IND sponsor, in collaboration with CPS/DTM, to include testing, processing, packing, labeling, or storage, or holding or distribution of the product, if the event meets the following criteria:

(i) Represents a deviation from current good manufacturing practice, applicable regulations, applicable standards, or established specifications that may affect the safety, purity, or potency of that product; or

(ii) Represents an unexpected or unforeseeable event that may affect the safety, purity, or potency of that product;

Cell product deviations will be reported as soon as possible but at a date not to exceed 45-calendar days from the date the PI/research team or CPS, DTM acquired information reasonably suggesting that a reportable event has occurred.

FDA reporting: All HCT/P deviations involving 361 cell products will be reported using MedWatch Form FDA 3500A according to FDA publication "Guidance for Industry: MedWatch Form FDA 3500A: Mandatory Reporting of Adverse Reactions Related to Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps).

12.5.2 Action Plan for Positive Results on Cell Product Safety Testing:

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

a) CPS, DTM personnel will notify the principal investigator, Dr. Dunavin (240-274-3599) (and pager 10682) or through the page operator. As soon as the identification and sensitivity report from positive sterility is available, a copy of the final report will be sent to the PI. The DTM QS officer and CPS Supervisor will determine the need for quality improvement based on the nature and extent of the incident.

b) If CPS is unable to reach Dr. Battiwalla within 15 minutes, - the page operator will be contacted (301-496-1211) to page the Hematology Branch Fellow on-call. NOTE: Dr. Barrett or

the Fellow on-call will contact the attending physician for the BMT Service, who will determine the extent of the work-up of a positive culture in consultation with staff from the Microbiology Service.

c) Dr. Dunavin/Barrett/Battiwalla/attending physician will discuss the positive results with the subject/parents, and specify the clinical therapy, antibiotic regimen and/or monitoring plan in consultation with staff from the Microbiology Service.

d) A contaminated sample of a product that has been administered to a subject will be handled in the same fashion as a serious adverse event, i.e., the PI or IND Sponsor will be responsible for notifying the NHLBI IRB, DSMB and the FDA. A full written report with description of events, laboratory findings, clinical evaluation and treatments would be submitted to the IRB within 15 working days. The PI or IND Sponsor will be responsible for filing this report with the FDA within 15 calendar days.

12.6 Protocol Monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/Monitors employed by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements of the Office for Human Research Protections (OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

13. HUMAN SUBJECTS PROTECTIONS

13.1 Rationale For Subject Selection

This protocol is open to pediatric and adult males and females from all ethnic and racial groups undergoing stem cell transplantation treatment protocols of NHLBI, NCI, NIAID, and NIDDK.

13.1.1 Competition with other Branch transplant protocols:

There are currently no active protocols for the treatment of subjects who do not respond to the standard therapies of these post-transplant complications.

13.1.2 Reimbursement for protocol participation, travel, food, and lodging

Compensation will not be given for participation in this study or for subjects' time and inconvenience. In determining reimbursement, the following factors are considered applicable to

this protocol: the subjects are diagnosed with a rare disease, the subject population is sick, the protocol offers the potential for direct benefit, the protocol regimen is demanding, and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required. No specific provisions for reimbursement are planned for this protocol since almost all subjects will be hospitalized and supported financially on existing stem cell transplant protocols.

13.2 Participation of Children, Pregnant Women and Decisionally Impaired

13.2.1 Pediatric subjects

Pediatric subjects will be included in this protocol. BMSC have been tested extensively in children and the NIH BMSC product has been tested in adults in a Phase 1 study and has been shown to be safe.

13.2.2 Pregnant women

Pregnant women will not be included in this protocol as pregnancy is a contraindication to the underlying transplant procedure and because of the unknown risks of the BMSC product to the fetus.

13.2.3 Decisionally-impaired

Patients who are decisionally-impaired will be included provided that they have a surrogate decision maker willing to provide informed consent.

13.3 Hazards/Discomforts

13.3.1 Related to the BMSC infusion

Serious complications or side effects from BMSC infusions have not been described. Infusion side effects have been minor including fever, hives, and nasal congestion.

13.3.2 Related to transplant-related complications.

Subjects in this trial may experience the spectrum of post-transplant complications described in detail in the associated protocols.

13.4 Risks/Benefits Analysis

13.4.1 For Transplant Subjects

According to previous studies using similar infusions, the risks of BMSC infusion appear to be low. As described in Section 2, *in-vitro* expanded BMSC have been used successfully to treat *de novo* and severe steroid refractory acute GVHD. In a recently published multicenter phase II study, 55 patients with steroid-resistant, severe, acute GVHD were treated with up to five infusions of BMSC at a median dose of $1{\cdot}4x10^6$ cells per kg bodyweight. HLA mismatched or unrelated HLA mismatched donors were used in most cases. Thirty patients had a complete response and nine showed improvement. No patients had side-effects during or immediately after BMSC infusions. Therefore, it is our expectation that this study presents greater than minimal risk but there is the potential for benefit in subjects enrolled. Stopping rules will apply if unexpected complications arise in this study.

For children, our expectation is that the research involves greater than minimal risk but presenting the prospect of direct benefit (Children's Risk Category Assignment: 2; 45 CFR 46.405).

13.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

13.5.1 Adults

The investigational nature and research objectives of this trial, the procedure and its 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. Participants eligible for this study may be very ill and incapable of providing consent at the time in which enrollment to this study is warranted, in which case a relative or legal surrogate would provide consent. All participants will have undergone transplant at NIH, and therefore, discussions introducing this study and potential participation in this study with possible need for surrogate consent will be held with potential candidates and their families, when warranted. Dr. Ito or any of the investigators listed on the cover page with consenting privileges will lead the discussion.

13.5.2 Informed Consent for adult participants unable to provide consent:

If there is an unexpected enrollment of a participant that is a patient unable to provide informed consent, the following justification and procedures per NIH HRPP SOP 14E will be used to enrolled participants in the this protocol.

Justification for inclusion: This research provides the prospect of direct benefit, therefore inclusion is justified.

Risk/Benefit Assessment:

This research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102)

Consent and Assent:

Procedures to determine capacity: If documentation of decision making capacity is not present in the medical record or the investigator questions the decision making capacity of the individual, then the Ability to Consent Assessment Team (ACAT) (301-496-9675 or 301-496-2429) will be contacted to make the determination.

Procedures for obtaining consent for legally authorized representative (LAR) (Risk Level B per SOP 14E): The following procedures will be followed starting with (1) in order to determining the LAR.

(1) For adults who cannot consent and have a court appointed guardian from a jurisdiction that allows it or a Durable Power of Attorney (DPA) for health care and/or research participation, the PI/designee or ACAT confirms appropriateness of surrogate to consent to research, including that:

(a)The surrogate understands that the protocol involves research;

(b)The surrogate understands the risks, potential benefits, (if any), and alternatives to the study;

(c)The surrogate has sufficient reason to believe participation in the study is consistent with the subject's preferences and values.

(2) Adults who cannot consent and who do not have a DPA or court-appointed guardian, but who are capable of understanding the DPA process and can assign a DPA, then ACAT confirms appropriateness of surrogate to consent to research, which includes assessing criteria (a)-(c) above.

(3) Adults who cannot consent, who do not have a DPA or court-appointed guardian, and who are not able to understand the DPA process to appoint a DPA, then a person at the highest level of the following list may serve as surrogate and authorize subject's participation if ACAT confirms surrogate appropriateness (which includes assessing criteria (a)-(c) above):

1. spouse or domestic partner;
2. adult child;
3. parent;
4. sibling;
5. other close relative

If at any time there is a question about the authority of the LAR to provide consent based on the jurisdiction appointing the durable power of attorney or other legal question regarding the LAR to provide consent, the Office of the General Counsel will be consulted.

Procedures to obtain assent and documentation of assent or dissent: The informed consent discussion will include the individual unable to provide informed consent along with LAR. The individual unable to provided informed consent will be asked if they agree to participate in the research and this will be documented in the medical record.

13.5.3 Informed Consent of Non-English Speaking Research Participants.

We anticipate the enrollment of non-English speaking research participants into our study. The IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2. If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2), and 21 CFR50.27 (b) (a). The summary that will be used is the English version of the extant IRB approved consent document. We request prospective IRB approval of the use of the short form for up to a maximum of 5 participants in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and that we will have that consent document translated into the given inherent language.

13.5.4 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), each of which must be addressed in relation to the protocol:

(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. This is an FDA-regulated study and as such, we are mandated to retain all samples, once collected, regardless of the age of the subject at the time of collection. 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.

13.5.5 New Study Related Information

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 subject participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

13.6 Conflict of Interest

A Guide to Preventing Financial and Non-Financial Conflicts of Interest in Human Subjects Research at NIH has been distributed to all NIH investigators who have reviewed the guide and indicated no conflicts of interest exist. No investigators hold patents or patents pending, or have any financial conflict of interest associated with this study.

14. PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

14.1 BMSC Infusion

14.1.1 Source

BMSC are an adherent, fibroblast-like cell population found in the bone marrow. Allogeneic BMSC for treatment can be grown from bone marrow aspirates or biopsies of normal donors, and are currently being used to treat a number of disorders, including graft-versus-host disease (GVHD), ischemic heart disease, peripheral vascular disease, and autoimmune diseases. The BMSC are produced in the Clinical Cell Processing Laboratory, located in the Cell Processing Section (CPS), Department of Transfusion Medicine (DTM), Clinical Center, NIH. Donors must be greater than or equal to 18 years of age, be afebrile, have normal hemodynamic parameters,

not be pregnant and must meet the screening requirements according to the policies and procedures of DTM. The HLA-type of the donor will be determined. Bone marrow is collected for generation of BMSC on protocol 10-CC-0053. Gentamicin is used in the processing of BMSC on 10-CC-0053 and thus allergy to gentamicin is specified as an exclusion criterion on this study. The collected cells are serially passaged into multilayer cell factories until approximately 300 to 900 million cells are generated. The cells are packaged into units of approximately 100 million cells and cryopreserved.

14.1.2 Toxicity

As previously noted, serious toxicities are not expected. Reported toxicities include fever, hives, and nasal congestion. Theoretically, transfusion reaction and allergic reactions are possible.

14.1.3 Formulation and preparation

Cells will be processed and prepared as per the BB-DMF filed by CPS, DTM with the FDA. Thawed BMSC suspended in Plasmalyte A will be given in an infusion and may be repeated twice weekly for four weeks followed by weekly for four weeks based on response. Each BMSC infusion will contain a target dose of 2 x 10^6 BMSC /kg (+/- 10%) viable BMSC (30-150 mL). Where possible repeat infusions will be obtained from the same donor but BMSC from other donors is permitted if same donor cells are not available. Donor sample identifiers will be tracked for each infusion.

14.1.4 Stability and Storage

The cryopreserved BMSC will be stored in the vapor phase of liquid nitrogen in the Cell Process Laboratory. This is a secure laboratory and the temperature and liquid nitrogen levels in the storage tanks are monitored continuously. The duration of time in which BMSCs retain their complete biological activity is not certain and a stability and viability testing plan is included in the CPS BB-DMF with the FDA. Previously BMSC have been stored in the Laboratory of Dr. P. Robey (NIDCR, NIH) for up to 15 years with retention of 90% viability upon thawing and hematopoietic progenitor cells have been stored even longer without evidence of loss of engraftment, proliferation, or differentiation potential. Data to date has demonstrated stability of the product for 4 hours post thaw; hence the expiration of the product will be considered, 4 hours post thaw.

14.1.5 Administration procedures

BMSC will be thawed rapidly in a 37˚C water bath and infused by IV infusion as per Section 3.4.

Appendix 1: Criteria for Diagnosis, Severity, Response and Eligibility for BMSC Trial

Consensus Conference Criteria[92]			
Extent of Organ Involvement			
	Skin	Liver	Gut
Stage			
1	Rash on <25% of skin[a]	Total bilirubin 2-3 mg/dL[b]	Diarrhea ≥ 500 mL/day[c] (child 10-19.9 ml/kg/day), or persistent nausea[d]
2	Rash on 25-50% of skin	Total bilirubin 3-6 mg/dL	Diarrhea ≥ 1000 mL/day (child 20-30 mL/kg/day)
3	Rash on >50% of skin	Total bilirubin 6-15 mg/dL	Diarrhea 1500 mL/day (child ≥ 30 mL/kg/day)
4	Generalized erythroderma with bullous formation	Total bilirubin >15 mg/dL	Severe abdominal pain with or without ileus
Grade[e]			
I	Stage 1 or 2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stage 2-4
IV[f]	Stage 4 or	Stage 4	-
(a) Use "rule of nines" or burn chart to determine extent of rash **(b) Range given as total bilirubin. Downgrade by one stage if an additional cause of elevated bilirubin has been documented.** **(c) Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade by one stage if an additional cause of diarrhea has been documented.** **(d) Persistent nausea with histological evidence of GVHD in the stomach or duodenum.** **(e) Criteria for grading given as minimal degree of organ involvement required to confer that grade.** **(f) Grade IV may also include lesser degree of organ involvement but with extreme decrease in performance status**			

Appendix 2: Criteria for Response to BMSC

Term	Definition
Complete response (CR)	Resolution of acute GVHD in all involved organs
Partial response (PR)	Organ improvement of at least 1 stage without worsening in any other organ system
Overall response (OR)	CR or PR
Mixed response (MR)	Improvement by at least 1 organ stage in at least 1 evaluable organ with worsening by at least 1 organ stage in at least 1 other organ
Stable disease	The absence of any clinically significant differences (improvement or worsening) sufficient to meet minimal criteria for improvement or deterioration in any evaluable organ
Worsening disease	Deterioration in at least 1 evaluable organ by 1 stage or more
No response	MR or stable disease or worsening disease

Appendix 3: Study Calendar for Early BMSC Pilot for Acute GVHD

Procedure	Protocol Screen	Day 0 +/- 24 hrs	Week 1	Week 2	Week 3	Day 28 +/- 72 hrs	Week 5	Week 6	Week 7	Day 56 +/- 72 hrs	Before and after each infusion +/- 24 hours[b, g]	3 weeks after last infusion +/- 7 days	Post Therapy Follow-up[e]
History	X												
Physical Exam	X												
Vital signs: BP, HR, RR, Temp, pulse ox	X												
Acute GVHD evaluation	X[a]	X	X	X	X	X	X	X	X	X		X	
Performance Score (KPS)	X												
Labs													
• Pregnancy Test[c]	X												
• CBC/differential	X											X	X
• Chemistry Panel	X											X	X
BMSC INFUSION[f]												X	
Correlative Research Studies[g]		X									X	X	
Response evaluation [d]						X				X		X	
Adverse events		X	X	X	X	X	X	X	X	X		X	X
Concomitant Medications		X				X				X		X	X

[a] Tissue biopsy if indicated: See Appendix 1for GVHD staging. aGVHD evaluation may occur in inpatient or outpatient setting and will occur at minimum on days of BMSC infusion (+/- 24 hours) and at any additional time that is determined necessary by the investigators. aGVHD evaluation will be done at least once weekly for the duration for the first 56 days of treatment.

[b] Monitoring according to NIH Clinical Center SOP: Administration of Products of Cellular Therapy

[c] In females of child-bearing potential

[d] Response assessment according to condition; response criteria in Appendix 2.

[e] Follow up between infusions and after the last BMSC infusion will be based on the subject's condition, as frequently as twice weekly.

[f] Premedicate 30-60 minutes prior to infusion. Infusions will be scheduled with DTM and given twice weekly no sooner than 48 hours apart (typically on Tuesday and Friday). The first dose of BMSC will be given within 120 hours of the first dose of corticosteroids. Infusion rate not to exceed 4-5 mL/min in subjects ≥35 kg, and infused over approximately 60 minutes in subjects < 35 kg (see Section 3.4).

[g] 54 mL of blood will be drawn [1 (6 ml) lavender top- K2-EDTA for plasma and 6 (8 ml) red-green "tiger top"] on Day 0 (pre BMSC infusion, +/- 24 hrs), Day 28 (+/-72 hrs), and Day 56 (+/- 72 hrs). 3 mL of blood [1 (3 ml) lavender top- K2-EDTA for plasma] will be drawn before and after each BMSC infusion (+/- 24 hours as long as before or after, respectively) and at 3 weeks after the last infusion (+/- 7 days).

Appendix 4: IRB Approved Hematology Branch Laboratory Research Studies

NHLBI HEMATOLOGY BRANCH 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)**		
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
C.24	Quantification of gene expression with RNA-seq	No	No
C.25	Characterization of chromatin and promoter/enhancer landscapes with ATAC-seq	No	No
C.26	Measurement of protein markers with SomaLogic's SOMAscan assay	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 inoculation 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

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