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Phase 2 Solid Tumor Immunotherapy Trial Using HLA-Haploidentical Transplant and Donor NK Cells: The STIR Trial

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I. INTRODUCTION

Although improvements in diagnosis, treatment, and supportive care have improved outcomes in patients with high-risk solid tumors, most still succumb to either treatment-related toxicities or, more commonly, progressive disease[1]. Novel chemotherapy and targeted agents have shown promise in early phase clinical trials, although durable disease remission appears to be the exception rather than the rule[2-8] . Such unsatisfactory results have prompted the design of new therapeutic strategies to treat these patients.

Cellular therapies have been explored as one way to treat high-risk solid tumors. Taking lessons learned from hematological malignancies treated with hematopoietic stem cell transplantation, non-hematopoietic solid tumors have also been shown to be amenable to graft-versus-tumor (GVT) effects. The ability of a new donor's immune system to abrogate disease progression would then provide a platform to achieve cancer control without additional intensive courses of chemotherapy or multiple rounds of high-dose radiation.

Natural killer (NK) cells are an important component of the innate immune system and have been shown to be potent mediators of GVT effects without contributing to graft-versus-host disease (GVHD)[9]. NK cells have been universally shown to be the first cellular emigrants to arise after allogeneic hematopoietic cell transplantation (HCT)[10], no matter which donor source is used. Adoptive infusion of donor NK cells have also been shown to deliver potent anti-tumor effects, with anti-cancer activity seen against a wide variety of solid tumors, including sarcomas, neuroblastoma, and brain tumors[11].

Taking advantage of the therapeutic effects of GVT from both transplantation of allogeneic hematopoietic stem cells and the infusion of donor NK cells, we are conducting a Phase II study using this dual cellular and adoptive immunotherapy approach to treat high-risk solid tumors. Using our experience with performing human leukocyte antigen (HLA)-haploidentical transplantation with *in vivo* T cell depletion using post-transplant cyclophosphamide (CY)[12], our multi-institutional expertise to manufacture clinical-grade NK cells under IND BB 13794, and our preliminary data demonstrating safety using a similar regimen in high-risk hematological malignancies (Protocol 2230), our Phase II study will evaluate the safety and efficacy of using this combined approach in treating patients with high-risk pediatric and adult solid tumors.

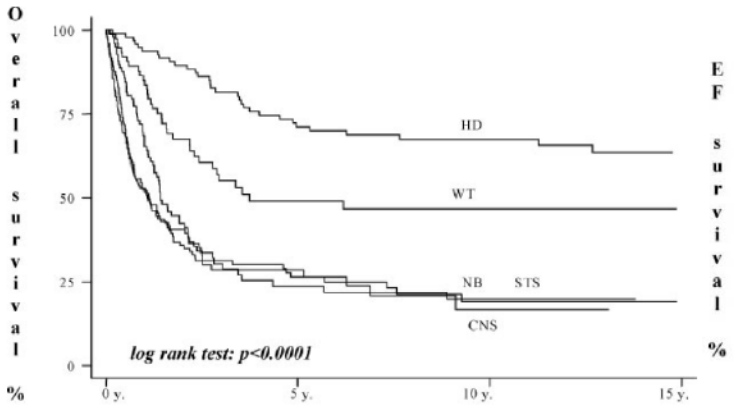
II. BACKGROUND

A. The Problem:

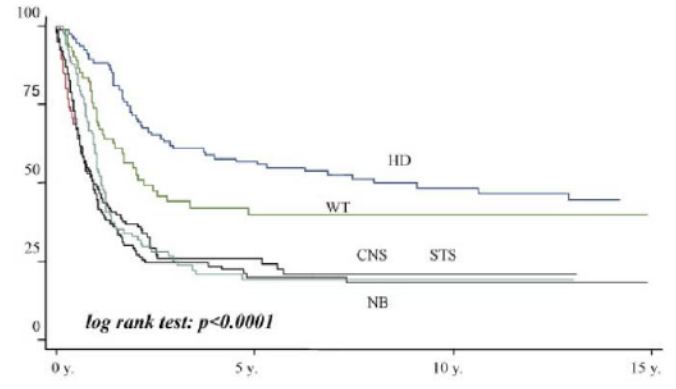
1) Solid Tumors Account for the Majority of Pediatric Cancer Deaths and
2) Relapsed/Refractory Solid Tumors Are Uniformly Fatal with Conventional Therapies

Data from the Surveillance, Epidemiology, and End Results 9 (SEER 9) registries, encompassing 10% of the US population, demonstrate that between 1975 to 2006, 60% of all pediatric cancer deaths were from solid tumors. This shows that although hematological malignancies are the most common form of pediatric cancer, the burden of pediatric cancer deaths greatly falls on patients diagnosed with non-hematopoietic solid tumors. Specifically, 25.7% of pediatric cancer deaths were from brain/central nervous system tumors, 15.5% from bone and soft tissue tumors, 9.1% from neuroblastoma, 2.7% from kidney tumors, 2.2% from liver and bile duct tumors, with the remainder of deaths attributed to solid tumors arising from gonads and other sites[13]. When evaluating outcomes in those patients who relapse after completion of therapy, the Italian Pediatric Off-Therapy Registry shows that 33% of patients with solid tumors who make it to the end of therapy without disease recurrence will still relapse within 5 months. Furthermore, the probability of long-term overall survival (OS) and event-free survival (EFS) in this group of patients differs significantly with the type of solid tumor, with Hodgkin Lymphoma (hematopoietic-derived solid tumor) leading OS over Wilms, neuroblastoma, soft-tissue sarcomas, and central nervous system tumors (**Fig 1**)[1], the latter 3 showing dismal survival. Specifically, overall survival was 38% (95% CI 33–42) at 5 years, 33% (95% CI 28–37) at

10 years, and 32% (95% CI, 27–36) at 15 years, with 5-year survival ranging from 71% in Hodgkins Lymphoma to 24% for soft-tissue sarcomas[1]. In patients with refractory tumors, outcomes are even more dismal. A retrospective study of 16 Phase I clinical trials treating pediatric refractory solid tumors at the National Cancer Institute between1992-2005 demonstrated that of 262 subjects, 17% achieved stable disease while only 4% had complete/partial response to the study drug; median overall survival was 5 months from the time of enrollment[14]. Although these early-phase studies were geared toward evaluating safety and feasibility of the approach rather than efficacy, this report suggests that durable disease response in these high-risk malignancies is still difficult to achieve even when using novel agents.



Survival probability (95% CI)			
HD	71 (61-79)	67 (57-76)	64 (52-73)
WT	49 (36-61)	47 (34-59)	47 (34-59)
NB	26 (19-35)	19 (11-28)	19 (11-28)
CNS	28 (20-38)	17 (8-29)	17 (8-29)
STS	24 (15-34)	20 (11-30)	20 (11-30)



Survival probability (95% CI)			
HD	57 (46-66)	49 (38-58)	45 (34-55)
WT	40 (28-52)	40 (28-52)	40 (28-52)
NB	20 (13-28)	18 (12-26)	18 (12-26)
CNS	26 (18-34)	21 (13-29)	21 (13-29)
STS	19 (11-28)	19 (11-28)	19 (11-28)

Fig 1. Overall Survival and Event-Free Survival in pediatric solid tumor patients who have relapsed after completing the end of therapy. Outcomes are dismal in patients with neuroblastoma (NB), soft-tissue sarcomas (STS), and central nervous system tumors (CNS) compared to Hodgkin Lymphoma (HD) and Wilms Tumor (WT).

B. Moving Beyond Autologous Transplants: Allogeneic Transplantation For The Treatment Of A Wide Variety of High-Risk Solid Tumors

With universal acceptance that most patients with relapsed and refractory solid tumors have poor prognosis, transplantation of allogeneic stem cells is regarded as an emerging and increasingly used treatment worldwide[15-17]. Unifying themes in using allogeneic transplantation for this set of diseases include attempting to enter transplant with low-burden disease to maximize efficacy of the immune therapy approach and minimizing toxicity by using reduced-intensity or non-myeloablative conditioning regimens, which would promote the emergence of mixed “donor-host” chimerism to promote GVT effects. Such a strategy would promote disease consolidation using a low-intensity immune approach after first undergoing some degree of cytoreductive therapy. This reduced-intensity approach also allows for improved immune reconstitution[18] and lower transplant-related mortality (TRM)[19] compared to a myeloablative approach in already heavily-treated patients.

There are multiple studies and case reports in the literature highlighting the use of allogeneic transplantation in the treatment of high-risk solid tumors. One group in Madrid performed HLA-haploidentical transplantation in 6 patients with a variety of high-risk solid tumors (neuroblastoma, Ewing sarcoma, a desmoplastic tumor, nasopharyngeal carcinoma, and embryonal rhabdomyosarcoma) and found response in all 6 patients, with 3 developing complete response (CR), 2 developing partial response, and 1 patient

having stable disease for greater than 9 months after reduced-intensity transplant. Three patients remain alive in CR at a median follow-up of 15 (4-65) months[20].

In neuroblastoma, *in vitro* and *in vivo* allo transplant murine models have established the "graft versus neuroblastoma" effect for this disease, showing improved tumor reduction after allogeneic transplantation compared to auto transplantation[21]. Lang et al from Tubingen, Germany developed a T/B cell depleted, NK cell enriched HLA-haploidentical protocol using a melphalan-based reduced intensity conditioning regimen to treat solid tumors. Four patients with neuroblastoma (NB) were included in this study of six patients [the other two being rhabdomyosarcoma (RMS), n=1, and Ewing sarcoma (EWS), n=1]. All patients entering transplant had significant tumor burden. Results showed that all had primary engraftment at a median of 11 days. One patient had secondary graft failure. Acute GVHD grade II was seen in four patients while two experienced limited chronic GVHD. Minimal transplant-related toxicity was observed and importantly, no TRM occurred. Four patients died from progression while two are alive, with an overall median survival of 6 (2-11) months at the time of the report[19]. Jubert et al.[22] reported on a pilot trial using unrelated cord blood to transplant children with refractory neuroblastoma using a reduced-intensity regimen. Despite using cord blood as the stem cell source, all patients engrafted. However, all patients also had disease progression at a median of 55 (26-180) days after transplant. The authors recommended that adoptive infusions of NK cells be used in future trials to help with early immune reconstitution to target disease more effectively post-transplant.

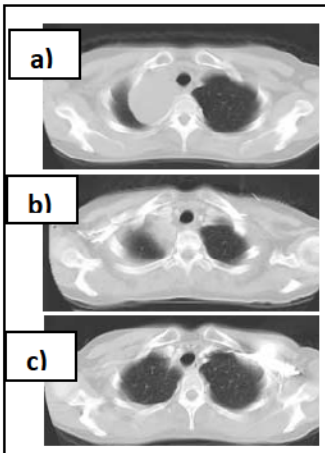


Fig 2. Regression of a primitive neuroectodermal tumor (PNET), a lesion within the Ewing sarcoma family of tumors, after undergoing allogeneic HCT. Shown is a right apical pulmonary tumor that has a) recurred after myeloablative autologous HCT, b) regressed after salvage chemo followed by reduced-intensity allogeneic HCT and c) achieved complete remission 8 months after HCT.

Sarcomas are another disease group for which allogeneic transplantation is being increasingly used with clinical evidence suggesting a potent "graft versus sarcoma" effect in both Ewing sarcoma[23-29], osteosarcoma[30;31], and rhabdomyosarcoma[32;33]. In the largest series of high-risk sarcomas treated with allogeneic transplantation, Baird et al. from the NIH evaluated 30 patients enrolled on trial, of which 23 eventually underwent reduced-intensity HCT[34]. No patients died within the first 100 days demonstrating that the regimen was well-tolerated in a heavily-treated population with all patients having rapid, early donor engraftment. Five of 23 patients remained alive (OS of 30% by Kaplan-Meier analysis at 3 years), including 3 of 7 (42%) transplanted without overt disease (median survival 14.5 versus 29.0 months from HCT). Koscielniak et al. reported long-term remission in a patient with Ewing sarcoma after HLA-haploidentical HCT[23]. Capitini et al. observed modulation of metastatic Ewing sarcoma after allo HCT[27]. Hosono et al. demonstrated a significant GVT effect in a patient with pulmonary Ewing sarcoma who failed high-dose chemo with autologous transplantation **(Fig 2)**[28]. In osteosarcoma, Fagioli et al. demonstrated initial regression of metastatic disease after non-myeloablative transplant although the patient relapsed later[30]. Goi et al. transplanted a 6 year old boy with diffusely metastatic disease. He developed grade III GVHD but eventually was weaned off all immunosuppression and is alive, relapse-free, at 67 months post-transplant[31]. Kounami et al[35] describe a patient with refractory osteosarcoma who was transplanted in complete remission (CR) 3. GVHD was induced by reducing immunosuppression when disease recurred. Initial results were promising suggesting a GVT effect, but was not durable and the patient eventually died at day +200 from rapidly progressive disease. In rhabdomyosarcoma, Ohta et al.[32] used a donor lymphocyte infusion to induce GVHD after allogeneic HCT in a patient with multiply-relapsed rhabdomyosarcoma to induce remission. At 31 months post-transplant, this patient remained disease-free. In a case-report, Donker et al. reports on a patient with stage IV rhabdomyosarcoma who at 4 years post-allogeneic transplant, remains in CR[33]. Misawa et al[36]. similarly showed in an adolescent patient with refractory rhabdomyosarcoma, partial remission was obtained after weaning immunosuppression drugs.

Anti-tumor effects have also been noted in many other non-hematopoietic high-risk solid tumors treated with allogeneic transplantation. Lundberg et al describe an adult with metastatic refractory medulloblastoma who achieved CR and is alive at 28 months post-transplant[37]. Secondino et al report on a 25 year old woman with recurrent, metastatic medullobastoma who was treated with a non-myeloablative conditioning regimen[38]. At 5 years post-transplant, she is alive, in CR, with chronic extensive GVHD. Abdel-Azim et al[39] report on a 2.5 year old boy with disseminated anaplastic large cell medulloblastoma who relapsed after multiple chemotherapy regimens and craniospinal irradiation. An autologous transplant could not be achieved due to inability to mobilize peripheral blood stem cells. An allogeneic transplant was used demonstrating full donor engraftment and mild GVHD. He relapsed at day +304, and biopsy revealed infiltration of donor CD3+ and CD56+ cells within the tumor. He eventually died from progressive disease at 2.5 years after transplant. This case demonstrates the promise of GVT and CNS infiltration of donor cells across the blood-brain barrier.

C. Approaches to Overcoming Graft-Versus-Tumor Delays in HLA-Haploidentical Transplantation: Immunosuppressive and Immunomodulatory Strategies

One problem when using allogeneic transplantation for the treatment of high-risk solid tumors is the delay of full GVT effects until donor immune reconstitution has occurred. This concept has plagued other trials, in which relapse or progressive disease frequently occurred within a short time of receiving the allogeneic transplant. Typically the patient is still receiving potent immunosuppression and has not yet achieved full donor engraftment. One strategy to improve the allogeneic response is to choose a donor who is HLA-mismatched to the patient. While this may predispose the patient to developing high-grade GVHD, several studies have shown that GVHD is protective against progressive disease (**Fig 3**). Thus in this trial, an HLA-haploidentical related donor will be the only acceptable donor for this study. Other strategies include immunomodulation of post-transplant immune suppression and using adoptive immunotherapy with donor NK cells to boost GVT effects.

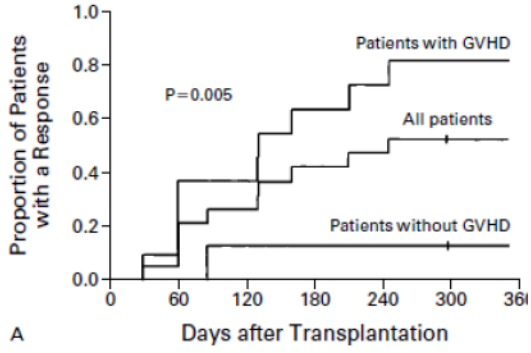


Fig 3. Patients with renal cell carcinoma who developed GVHD had more disease response

1. Immunosuppressive Strategy: Cyclophosphamide, Tacrolimus/Sirolimus, and Mycophelate Mofetil

a) Selective T Cell Depletion With Post-Transplant CY Will Not Affect Donor Stem Cells and Will Reduce GVHD: Pre-Clinical and Clinical Experience

It has been demonstrated since the 1970's that high doses of CY are not myeloablative, but are immunosuppressive due to its unique pharmacology. Both Jones[40] and Kastan[41] amongst others have studied aldehyde dehydrogenase, correlating high levels (found in hematopoietic progenitor cells) to CY resistance and low levels (found in T and B lymphocytes, NK cells) to CY sensitivity. Immature erythroid precursors express aldehyde dehydrogenase in intermediate levels. The first study demonstrating that CY's limiting toxicity is not hematopoietic was performed by Storb and colleagues[42] who showed that Rhesus monkeys receiving up to 200 mg/kg CY will have full hematological recovery without need for marrow

infusion. Brodsky et al.[43] have shown that in the clinical setting, doses of CY 200 mg/kg can be used to treat severe autoimmune diseases without impacting hematological recovery or needing autologous stem cell rescue. In the MHC-mismatched murine model, Luznik and colleagues[44] confirmed CY's ability to selectively deplete alloreactive, proliferating host and donor lymphocyte clones after using the FLU/TBI-based nonmyeloablative conditioning regimen developed at the Fred Hutchinson Cancer Research Center (FHCRC) in order to allow engraftment of mismatched stem cells. Further, work done at the NIH[45] has shown that in a fully MHC-mismatched murine transplant model, FLU and CY are synergistic and can successfully and preferentially deplete host T cells when compared to myeloablative doses of TBI. This suggests the potent ability of a FLU/CY regimen to promote engraftment without undue toxicity.

Based on a successful Phase I study conducted by O'Donnell and colleagues at Johns Hopkins University (JHU) (n=13)[46] Phase I study of HLA-haploidentical allogeneic HCT using post-transplant CY, a Phase II study was developed. Between 1999 and 2006, 68 patients with high-risk hematological malignancies requiring allogeneic HCT but lacking HLA-matched donors were enrolled on 2 nonmyeloablative HLA-haploidentical Phase II clinical trials at JHU and FHCRC[12]. These studies differed by immunosuppression only: the JHU regimen utilized 2 doses while the FHCRC regimen used 1 dose of CY after HCT. Twenty-five percent of all patients were ethnic minorities highlighting an important feature of using an HLA-haploidentical family member – the ability to provide a readily-available donor source to almost all patients. Conditioning consisted of CY 14.5 mg/kg/day (days -6 to -5), FLU 30 mg/kg/day (days -6 to -2), and 2 Gy TBI, followed by unmanipulated, HLA-haploidentical marrow allografts, with post-grafting immunosuppression consisting of CY 50 mg/kg (day +3 ± 4), MMF, and tacrolimus (both beginning 24 hours after the last dose of CY). MMF and tacrolimus are delayed to allow the full effect of CY in depleting alloreactive T cell clones to provide selective *in vivo* T cell depletion. Because of the use of HLA-haploidentical grafts, strong immunological barrier needed to be overcome with this regimen. Rejection occurred on average in 9 of 66 (13%) evaluable patients. Most patients converted to full donor chimerism by day +60. More notable was the cumulative incidence of grade III GVHD of 6% at 1 year with no grade IV GVHD. Non-relapse mortality (NRM) was 4% at day +100 and 15% at 1 year with infections being the leading cause. Two doses of post-transplant CY seemed to decrease rates of chronic GVHD. However, relapse was the primary contributor to low overall survival, with 58% of patients relapsing by 2 years after transplantation, leading to an overall survival of 36% by 2 years. The kinetics of relapse demonstrates early and continual disease-related mortality during the first year after HCT, as demonstrated by the incidence curve in **Fig 4**, supporting the need for anti-tumor intervention early after HCT. In this high-risk population that survives the initial risk of undergoing an HLA-haploidentical HCT, relapse continues to be the main cause of mortality. As in other protocols employing *in vivo* lymphocyte depletion to lower acute GVHD, relapse is a considerable cause of mortality. This supports the need for an early, preemptive intervention to facilitate GVT effects after HCT, which will be provided by donor NK cells in this current study.

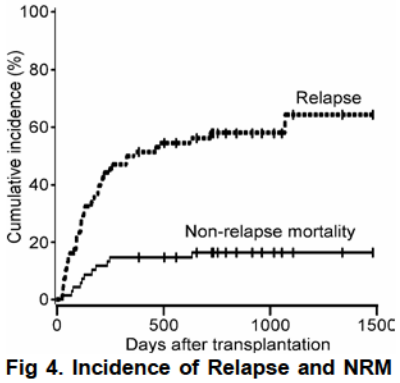


Fig 4. Incidence of Relapse and NRM with post-HCT CY approach. Kinetics or relapse suggests relapse often occurs early prior to full donor immune reconstitution

b) Effect of Tacrolimus and Mycophenolate Mofetil on NK Cell Function

Although NK cells have been shown to expand quickly after HCT, there is still some concern as to the effects of post-HCT immunosuppression on their function. In particular, calcineurin inhibitors such as cyclosporine (CSP) and tacrolimus, which are known to suppress T cell function, are of concern in NK cells due to their shared lymphocyte ancestry.

In the 1980's, it was shown that dog leukocyte antigen (DLA)-mismatched dogs receiving CSP alone had higher rejection rates than dogs receiving CSP with methotrexate, suggesting that residual host NK cells leading to graft rejection were not affected by CSP alone[47]. In a rodent model, CSP inhibited T cell but not NK cell responses[48]. Using human NK cells, the Minnesota group has further studied the effect of CSP on NK cells using in vitro assays and found that CSP induces a variety of unexpected and significant changes in the phenotype and function of cultured NK cells. CSP significantly inhibited the expansion of $CD56^{dim}$ cells and preferentially expanded $CD56^{bright}$ cells, which were found to be relatively resistant to CSP. However, it was also noted that a minority of these two populations could interconvert *in vitro* after a week of culture in cytokines and growth factors. Although CSP-treated NK cells showed more apoptosis compared to controls, the intracellular content of granzyme B, perforin, and surface expression of Fas ligand in NK cells incubated with CSP were not significantly different from controls. Additionally, higher percentages of IFN-γ producing NK cells were detected in CSP-treated cultures compared to controls. Mechanistically, CSP and tacrolimus work as a calcineurin inhibitors. Calcineurin, an integral component of lymphocyte activation, normally becomes triggered through an influx of calcium after cell stimulation. Calcineurin then dephosphorylates nuclear factor of activated T cells (NFAT), allowing NFAT to enter the nucleus for gene transcription to take place. In this study, CSP prevented dephosphorylation of NFAT. Surprisingly, however, NK cells cultured with CSP still had higher cytoxicity against K562 and Raji cells, suggesting that the effector function (granule release) is not dependent on the nuclear translocation of NFAT[49]. Thus, although there will be some immunmodulation of infused donor NK cells on this study, it is not expected that the NK cells will be adversely affected by tacrolimus.

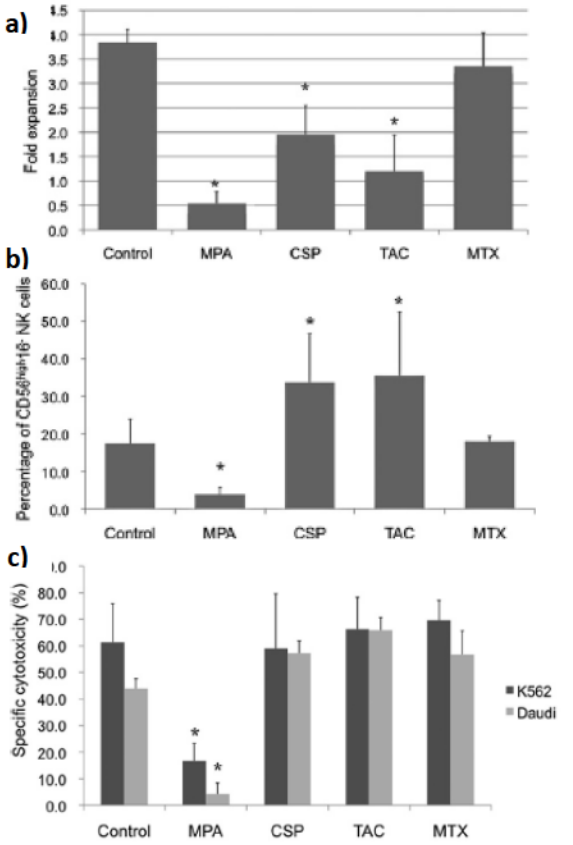


Fig 5. Effect of MMF on a) NK cell expansion, b) NK cell phenotype, and c) NK cell cytotoxicity demonstrating significant possible detrimental effects of MMF immunosuppression on NK cell functions.

MMF is another immunosuppressive drug being used after transplant in this trial. MMF is a prodrug that once metabolized, is converted to mycophenolic acid, which is a potent inhibitor of inosine monophosphate dehydrogenase. This enzyme results in decreased lymphocyte proliferation and recruitment to sites of inflammation, which may in part explain its potent T cell immunosuppressive properties[50;51]. MMF is a critical immunosuppressive agent used with particular emphasis in reduced-intensity conditioning trials and has strong track-record for promoting engraftment and controlling GVHD in this setting. However, Ohata and colleagues[51] studied multiple immunosuppressive drugs on NK cell activity and found that when comparing cyclosporine, tacrolimus, MMF, and methotrexate, MMF appeared to have the most significant effect on NK cell expansion (**Fig 5a**), phenotype (**Fig 5b**) and cytotoxicity (**Fig 5c**). Thus, for the purposes of this trial, MMF will be held until day +9 after HCT in order to fully take advantage of the GVT activity of adoptively-infused NK cells.

c) Use of Sirolimus for Both GVHD Prophylaxis and mTOR Inhibition

The mammalian target of rapamycin (mTOR) pathway is critical for many growth functions in cancer cells and is often upregulated in solid tumors. mTOR is also a key regulator of the phosphati-dylinositol-3-kinase (PI3K)-Akt signaling pathway and works as both an upstream mediator and a downstream effector of this pathway, thus promoting cancer cell proliferation while reducing cancer cell apoptosis. Thus, the ability to target mTOR in a treatment regimen for solid tumors is a promising therapeutic strategy. Derivatives of sirolimus (ie, temsirolimus, everolimus) are currently under investigation as single agents or in combination with other chemotherapy drugs to induce durable remissions in high-risk solid tumors[52;53].

Sirolimus is also a potent immunosuppressive drug and is being used as first-line prophylactic immune suppression after allogeneic HCT[54]. One of the benefits of sirolimus compared to tacrolimus and cyclosporine is its ability to induce proliferation of Tregs, a subset of T cells with potent immunosuppressive properties that have been shown to reduce the rates of GVHD[55]. There is also precedence for its use in the "post-transplant CY" regimen for immune suppression in patients with sickle cell disease, where tacrolimus was switched to sirolimus in the last 7 patients enrolled on this trial in order to decrease the risk of posterior reversible encephalopathy syndrome (seen in 3 of the prior 11 patients treated). Results showed extremely no acute or chronic GVHD in those patients receiving sirolimus[54]

Because of the anti-cancer and anti-GVHD benefits of sirolimus, and its prior precedence and safety profile when used after this particular transplant regimen, we will plan to switch patients from tacrolimus to sirolimus beginning at Day +28 after HCT. As sirolimus is only orally available, this timeframe will allow a consistent start after recovery from any mucosal irritation post-conditioning.

2. Immunomodulatory Strategy: Early, Prophylactic Infusion of Donor NK Cells To Boost GVT Effects

a) NK Cells Have Strong Anti-Tumor Effects In Diverse, High-Risk Solid Tumors

NK cells are emerging as a novel treatment in the field of adoptive immune therapy for a diverse and heterogeneous group of diseases, ranging from breast and ovarian cancers[56] to pediatric soft tissue sarcomas. With initial adoptive immunotherapy efforts focused on infusing T cells, NK cells are being increasingly studied by groups around the world. Two major benefits of NK cell immunotherapy over T cell immunotherapy include: 1) the lack of requiring prior antigen exposure and sensitization for initial function and 2) the inability to induce GVHD. Similar to T cells, NK cells also have the potent ability to fight infections and promote donor engraftment. Finally, the ability to infuse mature NK cells that have been educated in a healthy host has the potential to not only unleash a large GVT effect early post-transplant, but also bridge the profound immune deficiency gap that exists until donor engraftment occurs. Combined with early immune effectors that will emerge after allogeneic transplantation, NK cell adoptive immunotherapy has the great potential to augment GVT effects.

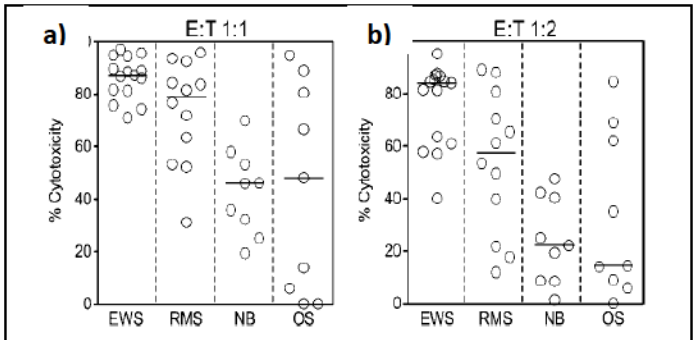


Fig 6. Ewing Sarcoma (EWS), rhabdomyosarcoma (RMS), neuroblastoma (NB), and osteosarcoma (OS) cell lines showing significant response to NK cell mediated cytotoxicity. Effector (E) to Target (T) ratios of 1:1 or 1:2 shown.

The current literature highlights multiple studies suggesting that NK cells can be manipulated to strike against a wide variety of solid tumors in both *in vitro* and *in vivo* settings. Neuroblastoma is one tumor that has been highlighted frequently as being susceptible to NK cell mediated killing[57-59]. Looking at a wide variety of tumors, Cho et al studied the effect of NK cells against neuroblastoma, osteosarcoma, Ewing sarcoma (EWS), and rhabdomyosarcoma cell lines. Although all cell lines tested showed potent NK-mediated GVT responses (**Fig 6**), the latter two diseases in this study showed the most anti-tumor response when co-cultured with NK cells. This response against human EWS was further confirmed in a murine NOD/SCID model injected with EWS tumor cells[11]. Studies into the mechanism of this response revealed that blocking NK activating receptors NKG2D and DNAX accessory molecule 1 (DNAM-1;CD226) inhibited this response suggesting the importance of these two receptors in initiating GVT.

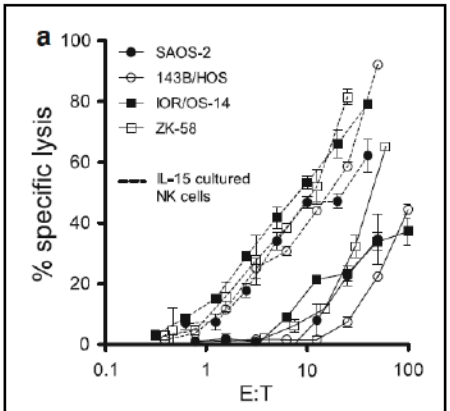


Fig 7. Osteosarcoma cell lines are amenable to lysis by freshly isolated (solid) and IL-15 cultured (dashed) NK cells. IL-15 was incubated with NK cells for 2 wks.

Verhoeven et al. also confirmed that NK cells lyse EWS cells through NKG2D and DNAM-1 activating receptor pathways[60]. Experiments showed that both resting and IL-15 activated NK cells were equally susceptible to lysis,that primary EWS cells express DNAM-1 and NKG2D ligands, and that cytotoxicity is dependent on activating receptor expression against these two ligands. Interestingly, NK cells taken from EWS patients at the time of diagnosis showed reduced cytotoxic activity despite normal NK numbers suggesting anergy and thus a tumor-evading mechanism for promoting cancer growth. This lack of NK activity could be reversed by adding IL-15 to the culture. These studies again suggest that NK cell adoptive immunotherapy could be an important therapeutic option in this set of tumors.

Osteosarcoma has been well-studied as a disease entity having significant response to NK-mediated cytotoxicity. An early report showed increased NK function and improved outcomes in osteosarcoma patients treated with IL-2[61]. Most recently, other groups have evaluated mechanisms of response to support this concept of NK vs osteosarcoma effects. Buddingh et al.[62] evaluated the ability of IL-15 activated NK cells to lyse osteosarcoma cell lines (**Fig 7**) and suggested again that the expression of NK-specific ligands on human tumor cells explained this mechanistic activity (**Fig 8**). In this study, researchers also showed that both chemosensitive and chemo-resistant osteosarcoma cells were amenable to NK cell-mediated killing.

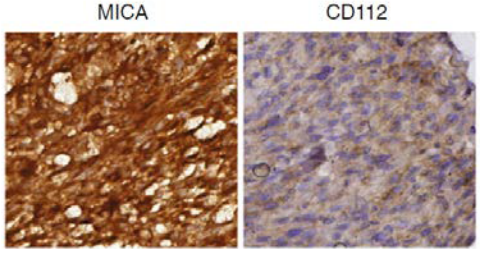


Fig 8. Human osteosarcoma cells express ligands for NK cell activating receptors NKG2D (ligand: MICA) and DNAM-1 (CD112) suggesting a mechanism by which NK cells kills osteosarcoma cells. Immunohistochemical staining shown.

Although initially thought of as an "immunologically privileged" site, the brain is now seen as a dynamic environment with the ability to host a multitude of immune cells and processes[63]Studies have demonstrated that immune cells have the ability to cross this barrier during both oncogenesis and inflammation[64;65],Thus, systemically-infusing donor immune cells appears to have rationale for treatment of CNS tumors assuming there is evidence of NK-mediated cytolytic activity for these diseases[66;67].

Human glioblastoma cells have been shown to be amenable to NK cell recognition and killing. Castriconi et al.[68] isolated cancer cells from glioblastoma samples taken from 9 patients. Ex vivo cultured glioma cells were efficiently killed by cultured NK cells through several explained mechanisms: low expression of HLA class I on glioma cells ("missing-self" recognition triggering NK cell killing), high levels of PVR (polio virus

receptor; CD155), and high levels of Nectin-2, the former two being ligands for DNAM-1, an important NK cell activating receptor as also mentioned earlier. Similarly, both CD133+ and CD133- medulloblastoma cell lines are killed through including NK-mediated activating receptors NKp46, NKp30, DNAM-1 and NKG2D[69].

There are a few potential unique safety concerns regarding infusion of allogeneic immune cells and their ability to cross into the blood-brain barrier. It has been reported in targeted chimeric antigen receptor immunotherapy (CAR-T trials), that a small number of patients have developed acute motor aphasia, which is reversible[70] There are also concerns of promoting inflammation within a closed environment (cytokine release syndrome), which can lead to seizures or increased intracranial pressure. Such responses, when seen, have been limited to highly-activated immune cells such as vaccine therapies[71] and CAR-T cells[70]. In terms of CNS-related immune toxicity risk when using the approach of NK cell adoptive immunotherapy for this trial, none of the patients on the predecessor trial described below Protocol 2230 (II.C.2.b) who have had prior CNS disease have had any adverse CNS events. Additionally, for this current trial, two patients with EWS who had active CNS disease had no adverse events, including no fevers, signs of CNS distress, motor deficits or encephalitis. Because the NK cells infused on these trials are not activated (unlike the other studies referenced), the likelihood of adverse inflammatory side effects are low. Additionally, strict stopping rules for toxicity are included in this trial.

b) Preliminary Data - Protocol 2230: Using Non-Myeloablative, HLA-Haploidentical HCT With NK Cell Infusion For The Treatment Of High-Risk <u>Hematological</u> Malignancies

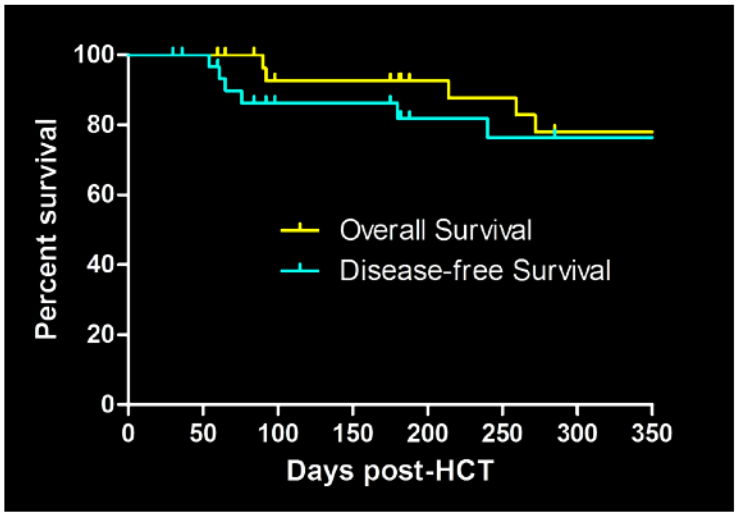
We developed a Phase I/II multi-institutional trial for high-risk hematological malignancies, adapting the non-myeloablative conditioning regimen published by Luznik et al.[12] and described in **Section II.C.1**, and added an infusion of donor NK cells on day +7 after transplant to boost GVT effects. In 32 heavily-treated patients treated to date on this actively-accruing study, cells were safely collected in all donors who provided both marrow stem cells for the transplant itself (day 0), as well as apheresis one week later (day +6), from which NK cells were derived from non-mobilized, peripheral blood mononuclear cells (PBMC). Additionally, safety was preserved in all patients who received donor NK cells on day +7, with no dose-limiting toxicities (DLTs) or other events noted. There have been no severe adverse events related to the NK cell infusion reported to the FDA. As part of this dose-finding protocol, two NK cells doses are being tested: 2.5 and 5.0 x 10^6 NK cells/kg. Both NK cell doses have been equally well-tolerated. In a subset of patients who were slated to receive the higher dose level, due to size discrepancy between donor and patient, the NK cell dose level was not achieved. However, all patients were able at a minimum to meet the lower defined limit of NK cells at 2.0 x 10^6/kg and have been counted toward the accrual and safety goals of this protocol to date. Results of the cell selection for the first 32 patients are listed in **Table 1**.

Table 1: Results of NK Cell Manufacturing Process (n=32) TNC: total nucleated cell

			Median (range)
Starting Product	**TNC x 10^9**		**16.4 (6.7-26.9)**
	NK x 10^9 **NK %**		**0.90 (0.21-2.47)** **5.4 (2.1-19.9) %**
	T x 10^9 **T %**		**7.94 (4.02-15.6)** **51% (32.5-69.2) %**
Final Product	**NK**	**Collected x 10^9**	**0.44 (0.14-1.27)**
		% Recovery	**54.3 (33.4-68.2) %**
		Max dose x 10^6/kg	**6.17 (1.95-20.5)**

		Dose Infused x 10^6/kg	5.0 (1.7-6.4)
	T	Collected x 10^4	4.27 (0.07-72.2)
		Dose Infused x 10^4 cells/kg	0.025 (0.001-0.85)
		Log T cell depletion	-5.41 (4.06-7.08)
	Final Viability		99.0 (94.5-99.8) %

Figure 9: 1 year Overall Survival and Disease-Free Survival in the first 32 patients treated on Protocol 2230



In terms of outcomes in the first 32 patients, there have been no NK infusional toxicities. Ten of 29 evaluable patients have developed acute GVHD, of which 7 developed grade II and 3 developed grade III. Six of 28 have developed chronic extensive GVHD. One patient, a 70 year old with multi-system organ failure, died of transplant-related causes. Eight of 32 patients have relapsed or progressed after transplant at a median of 125 days (range, 54 days – 1.5 years). Twenty-six of 32 patients are currently alive with a median follow-up of 280 days (range, 30 days – 4.2 years). Incidence curves for 1 year overall survival and progression-free survival can be found in **Figure 9**.

D. Approach to Overcoming Engraftment Failure and Rejection Without Adding Toxicity: The Benefit of Adding 3 Gy TBI

One concern when using allogeneic transplantation to treat non-hematopoietic solid tumors is the ability to promote durable donor engraftment. Unlike patients with treated leukemias and lymphomas who typically have poor marrow function and have little resistance to donor engraftment, solid tumor patients may be more resistant to engraftment when using reduced-intensity conditioning. Some patients may have only recently received a surgical intervention that spared marrow function, or be far-removed from prior cytoreductive chemotherapy. Thus, a strategy to promote engraftment and reduce rejection without increasing toxicity in this heavily pre-treated population is required for this protocol. Increasing radiation from 2 Gy to 3 Gy may be the solution to this problem. 3 Gy has been used successfully to augment non-myeloablative transplants that have rejected allografts that previously used 2 Gy of TBI[72]. In this study, Gyurkocza and colleagues studied 38 patients who had primary or secondary graft rejection underwent a second transplant using a reduced-intensity conditioning regimen that employed 3 or 4 Gy TBI. Day 100 non-relapse mortality was low at 5% suggesting that this was a well-tolerated regimen. 87% achieved successful sustained engraftment, with those having ongoing resistance to engraftment suffering from significant myelofibrosis. There was no difference in overall survival between patients receiving 3 Gy or 4 Gy (p=0.38). Thus, the strategy that will be used to promote engraftment and minimize toxicity on this study is to increase TBI from 2 Gy to 3 Gy

E. Rationale for Donor Stem Cell Source – Marrow vs Peripheral Blood Stem Cells

Marrow is the preferred and prioritized stem cell source for this protocol, as historically the transplant package of using an HLA-haploidentical donor with post-transplant CY was built around using bone marrow[12]. Recently, studies have shown that peripheral blood stem cells (PBSC) can be used just as effectively with no difference in GVHD, NRM, or toxicity compared to marrow and thus is noted to be a viable alternative to using marrow[73;74] Based on these more contemporary studies and results, the protocol will allow PBSC to be substituted for marrow in select cases (for example, if the recipient's weight exceeds donor weight such that a marrow harvest would not provide adequate stem cells safely). The goal CD34 dose will be 5.0 x 10^6/kg of recipient weight[73;74]. PBSC will be attempted to be collected during one leukapheresis procedure on day -1. If adequate cells are not collected, a second collection will be attempted on day 0. PBSC would be infused fresh on day 0. In select cases, cryopreserved PBSC may be used.

F. Manufacturing Highly-Purified, Clinical-Grade NK Cells

We have multi-institutional experience in manufacturing donor NK cells under IND 13794. Four normal volunteers between two sites (MCW and FHCRC), in addition to 10 donors for Protocol 2230 at the same two sites, have undergone non-mobilized apheresis to collect PBMC, from which highly purified NK cells are derived. As per our IND and prior experience, donor PBMC will undergo both a CD3 depletion to remove T cells followed by a CD56 selection to obtain NK cells (defined as CD56+/CD3- cells) using the Miltenyi CliniMACS™ system and Good Tissue Practices. Because safety and feasibility of NK cell production and infusion has been determined based on our initial study in high-risk hematological malignancies (Protocol 2230), **a goal of 5.0 x 10^6 NK cells/kg (*acceptable range: 2.0 – 8.0* x 10^6), with a safety limit of <10^4/kg of CD3+ cells, will be infused on this Phase II study**. This T cell safety limit is based on a dose-finding study performed by Lewalle et al. where DLI containing various doses of CD3+ cells were given prophylactically after T-cell depleted, myeloablative HLA-haploidentical HCT[75]. Should the CD3+ fraction of the product exceed this limit, the volume of the product will be reduced accordingly while maintaining the NK cell dose. NK cells will be released for infusion into the patient only after the following 3 criteria are met: 1) cell viability must be ≥70% 2) Stat gram stain must be negative 3) the number of residual CD3+ cells is <10^4/kg.

This IND allows for splitting the manufacturing of the NK cell product over 1 or 2 days. If performed over one day, the PBMC would be collected on day +6, CD3 depletion and CD56 selection would take place on day +7, and NK cells would be infused the same day (+7). If performed over two days, the CD3 depletion would take place on the day of PBMC collection (+6), stored overnight, and the CD56+ selection and infusion would happen the following day (+7) and infused later that day (+7). The preference would be to process and infuse as a one day process, ie, PBMC would be collected on day +6, and **NK cell selection and infusion would occur on day +7 after transplant**. Because of possible delays or scheduling concerns, NK cells may be infused as early as day +7 or as late as day +9 into the patient. All reagents and material used during processing and final formulation have been verified to be sterile and endotoxin free. Refer to the Chemistry, Manufacturing, and Control (CMC) data section of the IND for additional details regarding NK cell selection.

Initial validation runs were performed between MCW and FHCRC which provided the data required for approving the multi-institutional IND. Data shows that in four normal volunteer donors who did not receive G-CSF for mobilization, the CD56+ content of the collected PBMC was 0.58-1.13 (average 0.82) x 10^9. After CD3 depletion followed by CD56 selection, the following favorable results were seen: good NK recovery at 39-72.3% (average 54%), high NK purity at 93.4 ± 5.3%, very low T cell content at 0.006 ± 0.006%, and high $\log_{10}$ T cell depletion at 3.6 – 5.0 (average 4.8). NK cell selection in the first 8 donors on Protocol 2230 yielded similar results (**Table 1).**

G. Health-Related Quality of Life (HRQoL) Measures in Bone Marrow Transplant are Important Endpoints of Success

As intervention-based HCT research protocols are focused more and more on improving overall survival, an additional important component of gauging success is the ability to improve QoL in surviving patients. In our study, HRQoL will be evaluated at baseline, then at day +28, +100, +180, and 12 months post-transplant using validated surveys that have precedence for use in the pediatric[76] and adult[77] HCT setting: Child Health Ratings Inventory survey (CHRIs) for pediatric patients (validated in children: 5-12 yrs and adolescents: 13-18 yrs; parent proxy surveys validated for all patients, including children < 5 yrs) and the Functional Assessment of Chronic Illness Therapy (FACIT) scale[78;79] (specifically using Functional Assessment of Cancer Therapy - Bone Marrow Transplantation (FACT-BMT) and the Patient Reported Outcomes Measurement Information System (PROMIS)[80] scales for adult patients. For the purpose of our study, the CHRIs will be used in all pediatric patients treated at the Children's Hospital while the FACIT/PROMIS scales will be used for all adult patients treated at the adult hospital, regardless of patient age (triage plan recommended by Susan Parsons, PhD). These surveys evaluate concepts that include physical and emotional functioning, energy, body image, and worries. See **Appendix E** for details regarding survey psychometrics. Studies have shown that patients receiving reduced-intensity conditioning regimens and not having severe GVHD are important determinants in improving QoL[81;82], both of which are goals of this protocol design. Furthermore, in this set of high-risk solid tumors included in this trial, QoL measurements are an important tool to determine which of several competing and possibly equally-efficacious treatments are the most desirable for this high-risk patient population. Based on our low-toxicity experience with this transplant model in high-risk heme malignancies, we hypo-thesize that our intervention will improve HRQoL of surviving patients with high-risk solid tumors.

III. RATIONALE AND OVERVIEW OF STUDY

A. Rationale

We hypothesize that our Phase II cellular and adoptive immunotherapy study using HLA-haplo-identical HCT followed by an early, post-transplant infusion of donor NK cells on day +7 will not only be well-tolerated in this heavily-treated population (safety), but will also provide a mechanism to treat high-risk solid tumors, leading to improved disease control rate (efficacy), which is the combination of complete (CR) and partial (PR) response and stable disease (SD). We further propose that this infusion of donor NK cells will influence the development of particular NK and T cell subtypes which will provide immediate/long-term tumor surveillance, infectious monitoring, and durable engraftment.

B. Overview

Patients with high-risk solid tumors who have met eligibility will be enrolled on this trial for a goal enrollment of 20 patients over 4 years. Patients will receive a reduced-intensity conditioning regimen for 6 days that consists of FLU 150 mg/m^2, CY 29 mg/kg, and 3 Gy TBI, followed by HLA-haploidentical marrow (or PBSC) from a family member on day 0. On days +3 and +4, CY 50 mg/kg will be infused for selective *in vivo* T cell depletion. On day +5, tacrolimus will be started for ongoing post-grafting immunosuppression. MMF will be delayed until day +9 to take full advantage of NK cell alloreactivity. Non-mobilized peripheral blood mononuclear cells will be collected from donors on day +6, and the selected and enriched NK cell product will be infused into patients on day +7. Patients will be monitored for any transplant-related complications and will undergo disease monitoring every three months for the first two years post-transplant. Research studies will be conducted to follow the patient's immune status and quality of life post-transplant.

IV. OBJECTIVES

A. Primary Endpoint:

1. Disease Control Rate (CR + PR + SD) (6 months)

B. Secondary Endpoints:

1. Overall survival (1 year)
2. Progression-free survival (PFS) (6 months)
3. Non-relapse mortality (day +100)
4. Grades III-IV acute GVHD (day +100)
5. Response rate (CR + PR) (6 months) *[only in those patients who enter transplant with measurable disease]*

V. PATIENT SELECTION

A. Inclusion Criteria

1. No age restrictions
2. Only subjects who are not appropriate candidates for autologous or HLA-matched sibling transplants may be enrolled.
3. Diseases eligible
   a. High Risk Neuroblastoma (NB): Must have progressed on or recurred after standard frontline therapy including autologous HCT, or be ineligible for autologous HCT.
   b. Ewing Sarcoma Family of Tumors (EWS) [includes both bone and soft tissue Ewing and Peripheral Primitive Neuroectodermal Tumors (PNET)]. Must have progressed on or recurred after standard frontline therapy which includes doxorubicin and ifosfamide.
   c. High-Risk Rhabdomyosarcoma (RMS) or Intermediate Risk Alveolar RMS recurring as more than loco-regional tumor: Must have progressed on or recurred after standard frontline therapy which includes chemotherapy with vincristine, actinomycin, and cyclophosphamide AND either surgery or radiotherapy.
   d. Osteosarcoma: Must have progressed or recurred after standard frontline therapy. If first relapse, must have recurred with a) ≥ 4 lung nodules; b) bilateral lung involvement; or c) relapse outside the lungs.
   e. CNS tumors: High risk malignant brain tumors that are recurrent or refractory to standard frontline therapy are eligible. Diagnoses include: Medulloblastoma, primitive neuro-ectodermal tumor (PNET), ependymoma, high grade (grade 3 or 4) glioma/astrocytoma, germ-cell tumor, or atypical teratoid-rhabdoid tumor (ATRT)

B. Exclusion Criteria

1. Rapidly-progressing disease prior to HCT, defined as clinical or radiographic evidence of disease progression ≤ 3 weeks prior to protocol registration despite previous achievement of stable or no disease **(Appendix C & D)** (Note: after disease eligibility has been determined, additional imaging studies are not necessary during the three weeks before the start of conditioning unless there are clinical concerns).
2. Patients who have reached radiation threshold limits and are excluded from receiving 3 Gy TBI.
3. Diffuse intrinsic pontine gliomas (DIPG) are excluded.
4. Performance status: Karnofsky or Lansky <60%
   Note: Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score
5. Patients, who in the opinion of the investigator, may not be able to comply with the treatment plan or safety monitoring requirements of the study

6. Significant organ dysfunction that would prevent compliance with conditioning, GVHD prophylaxis, or would severely limit the probability of survival, defined as:
   a. Cardiac: *For patients not taking inotropic medications and who do not have cardiac failure requiring therapy:* Symptomatic coronary artery disease or ejection fraction <35% or, if unable to obtain ejection fraction, shortening fraction of <26%. If shortening fraction is <26% a cardiology consult is required with the PI having final approval of eligibility. *For patients taking inotropic medications:* Patients displaying corrected cardiac function will be eligible, i.e., patients who take inotropic medications to maintain EF ≥ 35% and SF≥ 26% cardiac function eligibility.
   b. Pulmonary: DLCO <40% TLC <40%, FEV1 <40% and/or receiving supplementary continuous oxygen
   c. Liver: Patient with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, bridging fibrosis, and the degree of portal hypertension. The patient will be excluded if he/she is found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3mg/dL, or symptomatic biliary disease
7. Patients with serious active infections
8. HIV seropositive patients
9. Patients with poorly controlled hypertension despite multiple antihypertensive medications
10. Fertile females who are unwilling to use contraceptive techniques during and for the twelve months following treatment, as well as females who are pregnant or actively breast feeding
11. Fertile males who are unwilling to use contraceptive techniques during and for the twelve months following treatment
12. Life expectancy severely limited by diseases other than malignancy
13. Patients who have received a prior allogeneic HCT are ineligible

VI. DONOR SELECTION

An Unrelated Donor Search is not required for entry on this trial.
Lack of HLA-matched related or unrelated donors is not a requirement for entry on this trial.

A. Inclusion Criteria

1. Related, HLA-haploidentical donors who are identical for one HLA haplotype and mismatched for any number of HLA-A, -B, -C, DRB1 or DQB1 loci of the unshared haplotype.
2. Marrow will be prioritized as the hematopoietic stem cell source of choice. In cases where adequate stem cells cannot be collected, fresh (preferred) or cryopreserved donor PBSC may be substituted. In the case that PBSC are used, the donor must be 18 years of age or older.
3. HLA-haploidentical donor selection will be based on standard institutional criteria; otherwise no specific prioritization will be made amongst the suitable available donors.

B. Exclusion Criteria

1. Children less than 12 years of age (marrow) or less than 18 years of age (PBSC).
2. Children greater than or equal to 12 years of age who have not provided informed assent in the presence of a parent and an Attending physician who is not a member of the recipient's care team
3. Children greater than or equal to 12-17.9 years of age who have inadequate peripheral vein access to safely undergo apheresis
4. Donors unable or unwilling to undergo marrow harvest or PBSC collection for the initial HCT, storage of autologous blood prior to marrow harvest (if applicable), or apheresis one week after marrow harvest

5. Donors who are not expected to meet the minimum target dose of marrow cells (1 x 10^8 total nucleated cells/kg recipient weight) for the initial marrow HCT or PBSC transplant (5.0 x 10^6 CD34/kg recipient weight). *The average nucleated cell content of harvested marrow is 22 x 10^6 nucleated cells/mL or 220 x 10^8 total nucleated cells/Liter*
6. HIV-positive donors
7. Donors who are cross-match positive with recipient

VII. INFORMED CONSENT

Patients with high-risk solid tumors will be referred for consideration of HCT. Both patient and donor will be completely evaluated. The protocol will be discussed thoroughly with patient, donor and family, and all known risks to the patient and donor will be described. The goals of the study, requirement for data collection, and requirement for release of medical records will be discussed with the patient and/or his/her parent/guardian. Alternatives to care will be discussed. All potential risks associated with the use of FLU, low dose TBI, CY, immunosuppressive drugs, allogeneic HCT with an HLA-haploidentical donor, and NK cell infusion from the same HLA-haploidentical donor, will be discussed as objectively as possible with the patient. Discussion of potential complications will include graft rejection, GVHD, infections, and death. Regimen-related complications and risk of relapse will be discussed. With the donor, discussion about the potential risks of marrow or PBSC donation, autologous infusion of packed red blood cells (if applicable), and apheresis collection will be discussed. Donors who are children greater than or equal to 12 years of age and less than 18 years of age must willingly sign the assent form in the presence of the parent and an Attending physician independent of the recipient. If there is any question as to the ability of the donor to provide informed assent, another donor must be used.

Informed consent from the patient and donor will be obtained using forms approved by the local institution's Institutional Review Board (IRB). Informed consent will be obtained by the Principal Investigator, Co-Investigator, or Attending Physician who is familiar with the study but not necessarily an investigator. When patients are less than 18 years of age, consent will be obtained from parents or legal guardian(s). Patient and donor assents will be obtained according to institutional guidelines where appropriate. A summary of the conference will be documented for the medical record detailing what was discussed. A copy of the signed consents will be given to the patient and donor.

VIII. PROTOCOL REGISTRATION

Eligible patients will be referred to the Bone Marrow Transplant Intake Coordinator for arranging consultation. Patients will be enrolled on study between 9 am-4 pm, Monday through Friday. Urgent registrations will be made upon the discretion of the Principal Investigators of this study.

IX. PLAN OF TREATMENT

A. Disease Status and Reduction Prior to Transplant

Tumor reduction should be attempted prior to transplant using chemotherapy, surgery and/or radiation therapy.
Presence of residual tumor is not required prior to study entry. If no measurable disease is present prior to transplant, tumor response will be judged by ongoing lack of measurable disease on follow-up imaging.

B. Recipient Treatment Plan

All pediatric patients will be admitted for the conditioning regimen and will stay inpatient until engrafted and other medical issues can be maintained as outpatients. Adult patients may undergo conditioning and post-

transplant treatments according to their institutional standards. If adult patients must initiate tacrolimus as a continuous infusion rather than IV bolus, they must be admitted until IV bolus/PO formulations can be used. Per the FDA's requirements, Phase II donor NK cell infusions can be limited to an observation period of 2 hours after infusion is completed.

Menstruating female patients should be placed on an anti-ovulatory agent prior to initiating the conditioning regimen.

See **Figure 10** for details of the treatment regimen.

1. Conditioning Regimen

	Days					
Conditioning	**−6**	**−5**	**−4**	**−3**	**−2**	**−1**
CY 14.5 mg/kg (no MESNA)	•	•				
FLU 30 mg/m²/day	•	•	•	•	•	
TBI 300 cGy						•

[5] **NK cell dose: 5.0 x 10^6 CD56+CD3-/kg**
Acceptable range:
2.0 - 8.0 x 10^6CD56+CD3-/kg

HCT (Priority given to marrow as preferred stem cell source over mobilized PBSC)	**0**

Post-Transplantation	**+3**	**+4**	**+5**	**+7**	**+9**	**+28**	**+40**	**+84**	**+180**
CY 50 mg/kg	•	•							
MESNA (dosed 100% CY dose)	•	•							
Tacrolimus (refer to XI.E for dosing) – goal level 5-15 ng/mL			Start	→	→	Switch[1]			
MMF[1] (refer to XI.F for dosing)					Start[2]		Taper[3]	Stop[3]	
G-CSF 5µg/kg/day			Start[4]						
Sirolimus (refer to XI.G for dosing) – goal level 5-15 ng/mL						Start		Taper[3]	Stop[3]
Donor NK Cell Infusion[5]				•					

Fig 10. HLA-Haploidentical, Reduced-Intensity HCT with Donor NK Cell Infusion Treatment Plan
Conditioning consists of CY 29 mg/kg, FLU 150 mg/m², and 300 cGy TBI. Bone marrow or PBSC are infused on Day 0. G-CSF begins day +5 after marrow or PBSC infusion and continues until engraftment is seen (ANC >500 x 3 days). Post-transplantation immunosuppression consists of CY (day +3, +4), tacrolimus→ sirolimus, and MMF. Tacrolimus must begin at least 24 hours after the CY infusion (day +5) in order to prevent blunting of the alloreactive response needed for effective CY targeting of proliferating lymphocytes.
[1] **Tacrolimus will be switched to sirolimus between days +28 and +35 if patient can tolerate oral medications.** The method to transition tacrolimus to sirolimus will be per attending physician discretion and will require short overalap of both drugs until adequate sirolimus levels are attained.
[2] **MMF is always started on day +9**, regardless if day +7 NK cell infusion is delayed until day +8 or day +9.
[3] **Based on GVHD Status, MMF and sirolimus will be tapered and discontinued**. Tapers may occur more rapidly in the absence of GVHD and/or presence of ongoing/progressive disease based on clinical judgment only after discussing with study PI.
[4] Continue G-CSF until ANC>500 x 3 days.
[5] A single infusion of donor NK cells at a dose of **5.0 x 10^6 (range, 2.0 – 8.0) CD56+/CD3- cells per kg** is given on Day +7. Allowable schedule for infusion: day +7 to +9. All attempts will be made to infuse on day +7. Patients must not be receiving prednisone equivalent dose ≥ 0.25 mg/kg from 1 day before until 2 days after NK cell infusion unless clinically necessary.

a. Fludarabine

i. Fludarabine dose is based on estimated creatinine clearance (**Table 3**)

Adults: creatinine clearance may be estimated by the Cockcroft Formula:
CLcr = [(140-age) x weight (kg) x 0.85 (for women only)]/[72 x creat (mg/dl)].

Pediatrics (Age <17): Formal measurement such as iothalmate study

ii. Fludarabine is administered by IV infusion over 30 minutes from days -6 to -2
The dose of fludarabine is based on m^2 and will always use Actual Body Weight

For patients having any prior CNS disease, any prior brain radiation, or any prior intrathecal chemotherapy, the fludarabine dose should be reduced by 20%.

Table 3: Fludarabine Dose Based on Creatinine Clearance

Creatinine Clearance mL/min	Daily Fludarabine Dose (mg/m^2)
> 60	30
46-60	24
31-45	22.5
21-30	19.5
<20	15

b. Cyclophosphamide

i. Cyclophosphamide is administered by IV infusion over 1 hour on days -6 and -5 with IV fluid administration
Refer to standard practice guidelines for administration guidelines. Patients will be monitored closely for urine output and hematuria. MESNA is not required at this dose.

ii. Cyclophosphamide is dosed according to the Adjusted Body Weight if the patient's Actual Body Weight is greater than 125% of the Ideal Body Weight (IBW).

For both Adult and Pediatric Patients, Adjusted Body Weight will be calculated as follows:
Adjusted Body Weight = IBW + 0.25 (Actual Weight – IBW)

If the patient is underweight, ie, the IBW is greater than the Actual Body Weight, then Actual Body Weight will be used

c. Total Body Irradiation

300 cGy TBI will be given on day –1 at a rate of 6-15 cGy/min per radiation oncology standard guidelines.

2. Hematopoietic cell infusion

Fresh marrow will be prioritized as the donor stem cell product of choice. In cases where adequate stem cells cannot be collected from marrow, fresh donor PBSC will be substituted. Cryopreserved PBSC may also be used. Thus the preference will be fresh donor marrow > fresh donor PBSC > cryopreserved donor PBSC.

a. Bone Marrow

Donor marrow will be harvested with a target yield of 4 x 10^8 nucleated cells/kg recipient body weight. This volume must not exceed 20 mLs/kg donor weight. The minimum acceptable yield should be 1 x 10^8 nucleated cells/kg recipient IBW. **Marrow collection final volume goal should be based on the mid-collection total nucleated cell count.** The composition of allografts will be determined by flow cytometry.

Products from donors with major or minor red blood cell (RBC) incompatibilities will be processed for RBC and plasma reduction according to standard program policies and procedures.

b. Peripheral Blood Stem Cells

In cases where it has been deemed that donor marrow harvest cannot be accomplished, G-CSF-mobilized PBSC will be collected from the donor. The donor will receive 5-7 days of filgrastin (G-CSF) subcutaneously from days -6 or -5 through day -1. Dosing of G-CSF will be per standard institutional procedures for normal volunteer donors and may be needed for up to 7 days. **The target cell dose will be 5.0 x 10^6 CD34/kg of recipient weight.** If this target cannot be met, a 6th dose of G-CSF will be given on day 0 and a second leukapheresis procedure will be conducted. Cells will be combined to give the target dose and infused fresh on day 0. Any leftover cells will be cryopreserved for later clinical use, such as donor lymphocyte infusion. In certain cases, only cryopreserved PBSC may be available for use. The target dose will be 5.0 x 10^6 CD34/recipient weight based on post-thaw calculations and infused on day 0.

3. Post-transplantation Immunosuppression

Immunosuppression to permit engraftment and provide GVHD prophylaxis will be performed with Cyclophosphamide (CY), Mycophenolate Mofetil (MMF) and Tacrolimus.

a. Cyclophosphamide

On **Days +3 and +4**, CY will be given as a single dose of 50 mg/kg IV

Cyclophosphamide is dosed according to the Adjusted Body Weight if the patient's Actual Body Weight is greater than 125% of the Ideal Body Weight (IBW).

First dose of post-transplant CY will be given within 48-72 hours of HCT infusion as a 1 hour infusion with MESNA prophylaxis and IV hydration. Refer to standard practice guidelines. Urine output and signs of hematuria will be monitored closely. *To maximize the effectiveness of post-transplant CY, it is critical that immunosuppressive agents are to be avoided FROM THE MORNING OF STEM CELL INFUSION until 24 hours AFTER the completion of the post-transplant CY unless there is medical necessity. This includes corticosteroids as anti-emetics.*

b. Tacrolimus

i. Starting on **Day +5** (24-36 hours after last dose of CY), tacrolimus will be given at a dose of 0.03 mg/kg/day (for patients <30 kg) continuous infusion over 22-24 hours or 1 mg/day (for patients >30kg) IV once a day over 1-2 hr.
Tacrolimus is dosed based on Adjusted Body Weight. Tacrolimus should be changed to an oral dosing schedule once oral medications are tolerated and once a therapeutic level (5-15 ng/ml) is achieved. Oral tacrolimus is dosed twice daily. Serum levels of tacrolimus should be measured on day +8 and then a minimum of twice weekly thereafter and the dose adjusted accordingly to maintain a level of 5-15 ng/ml.

Tacrolimus will be switched to sirolimus at **day +28** (window until day +35) as long as the patient can tolerate oral medications. During this period of transition, there will be a short overlap between concomitant tacrolimus and sirolimus dosing. This short overlap with concurrent administration of both drugs has been shown to be well-tolerated with minimal effect on drug pharmacokinetics[83;84].

ii. Guidelines for Tacrolimus Dose Adjustment and Monitoring

a) If there is nausea and vomiting which prevents oral intake at any time during tacrolimus treatment, the drug should be given intravenously at the appropriate dose that was used to obtain a therapeutic level. (IV: PO ratio = 1: 4).

b) It is recommended that whole blood trough levels of tacrolimus (i.e., just prior to the next dose) should be obtained on Day +8 and then a minimum of twice weekly until the taper is initiated, unless high levels (>20 ng/ml) are detected or toxicity is suspected, in which case more frequent monitoring will be performed as clinically indicated. The dose should be adjusted accordingly to maintain a level of **5-15 ng/mL**.

c) Dose reductions should only be made if tacrolimus toxicity is present or levels exceed 20ng/ml in the absence of toxicity. Dose reductions of tacrolimus for high levels without toxicity should be conservative, e.g. 25%, to avoid inadequate immunosuppression. If creatinine is greater or equal to 2 times the baseline level then the tacrolimus dose will be reduced by 25%.

d) Blood pressure, renal function tests (creatinine, BUN), electrolytes and magnesium need to be followed at least two to three times per week while receiving tacrolimus to full dose and then twice weekly or per attending until tacrolimus is discontinued

e) Tacrolimus levels should be performed more frequently when 1) drug is converted from oral to IV or IV to oral, 2) dose adjustments are made due to levels outside the therapeutic range, or 3) voriconazole (see table below) is initiated or withdrawn 4) if toxicity is suspected. Steady state levels will not be achieved for at least 72 hours after any change in dosing, i.e. levels determined earlier may not reflect an accurate steady state concentration.

f) Patients requiring hemodialysis should have tacrolimus levels maintained in the therapeutic range (5 to15 ng/ml).

g) Grapefruit and grapefruit juice affect metabolism of tacrolimus and should be avoided. Oral tacrolimus should be taken consistently with or without food. Refer to **Table 4** for other interactions.

Table 4: Medications that Affect Tacrolimus and Sirolimus Levels

Decrease Tacrolimus Levels	Increase Tacrolimus Levels
Dilantin	Steroids
Phenobarbital	Fluconazole
Carbamazepine	Ketoconazole
Rifampin	Itraconazole
Caspofungin	Voriconazole*
	Cimetidine
	Macrolide antibiotics
	Calcium channel blockers
	Danazol
	Metoclopramide
* When initiating therapy with voriconazole in patients already receiving tacrolimus, it is recommended that the tacrolimus dose be reduced to one-third of the original dose, (a 67% reduction) and followed with frequent monitoring of the tacrolimus blood levels. Increased tacrolimus levels have been associated with nephrotoxicity. When voriconazole is discontinued, tacrolimus levels should be carefully monitored and the dose increased as necessary.	

c. Mycophenolate Mofetil

i. Starting on **Day +9**, MMF will be given orally at a dose of 15 mg/kg based on adjusted body weight every 8 hours. ***MMF will always begin on day +9, regardless if NK cell infusion is delayed until day +8 or +9.***

MMF will be **tapered after day +40** (adapted dose-reduction to be **discontinued by day +84**) if there is no evidence of GVHD. *More rapid taper could be considered based on disease status only after discussing with the PI.*

ii. Guidelines for MMF Dose Adjustment and Monitoring
   a) Initiating MMF therapy: Oral administration of MMF will be at 15 mg/kg orally every 8 hours (45 mg/kg/day) starting on Day +9. If there is nausea and vomiting at any time preventing the oral administration of MMF, MMF should be administered intravenously at the appropriate dose.
   b) Maintaining MMF: Markedly low (<40%) donor T-cell chimerism after HCT may indicate impending graft rejection. MMF should be continued at full dose or, if the MMF taper has been initiated, reinstitution of full dose MMF should occur. If MMF has been discontinued, MMF should be reinitiated at full dose.

iii. Guidelines for MMF Dose Adjustment Based on Toxicity
   a) If in the clinical judgment of the attending physician the observed toxicity is related to MMF administration, a dose adjustment may occur. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF)
   b) Gastrointestinal Toxicity. Severe gastrointestinal toxicities such as gastrointestinal hemorrhage have been very rare after nonmyeloablative HCT. In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea that is considered to be due to MMF after day 28, a 20% dose reduction will be made or the drug may be given IV. If severe refractory diarrhea or overt gastrointestinal bleeding occurs, MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.
   c) Neutropenia. Based on previous experience in patients after nonmyeloablative HCT, dose adjustments are likely to occur because of hematopoietic adverse effects, in particular neutropenia. A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications. If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for severe, prolonged neutropenia (ANC <500/ul for 5 days or more) that persists after day +21 post-transplant. Dose reductions should be conservative (20%). After day +21, the use of G-CSF will be permitted for severe neutropenia. For severe hematological toxicity related to MMF (neutropenia > 5 days refractory to G-CSF), MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and all CRFs.

d. Sirolimus

i. Starting on **Day +28** (window: day +28 to +35), Sirolimus will be started at a dose based on BSA (see below). The dose should be adjusted accordingly to maintain a level of **5-15 ng/ml**. If patients cannot tolerate oral medications for any reason, the transition of tacrolimus to sirolimus will be delayed until oral medications can be taken.

Patients with BSA > 1.5 m^2: Sirolimus will be started on day at 2.0 mg every day orally.

Patients with BSA < 1.5 m^2: For children and patients with BSA of < 1.5 m^2, the dose will be based on BSA as follows: started at 1 mg/m^2/day to be rounded at the nearest 0.1mg.

In the absence of GVHD, Sirolimus should be tapered beginning at **day +84** and discontinued on **day +180**. In the presence of GVHD or if the patient is receiving glucocorticoid therapy, continuation of sirolimus will be at the discretion of the attending physician.

When first beginning sirolimus, there will be a short overlap between concomitant tacrolimus and sirolimus dosing. This short overlap with concurrent administration of both drugs has been shown to be well-tolerated with minimal effect on drug pharmacokinetics[83;84].

ii. Guidelines for Sirolimus Dose Adjustment and Monitoring[85]

a) To minimize variability of exposure to sirolimus, the drug should be taken consistently with or without food. Grapefruit juice reduces CYP3A4-mediated metabolism of sirolimus and should not avoided. See **Table 4** for additional medications that affect sirolimus metabolism.
b) It is recommended that whole blood trough levels of sirolimus (i.e., just prior to the next dose) should be obtained 1-2 days after starting drug then a minimum of twice weekly until the taper is initiated, unless high levels (>20 ng/ml) are detected or toxicity is suspected, in which case more frequent monitoring will be performed as clinically indicated. The dose should be adjusted accordingly to maintain a level of **5-15 ng/ml**.
c) Dose adjustments are based on clinical toxicity, blood levels, and GVHD. For levels <5 ng/mL, the dose is increased by increments of 25% until the target range is achieved. Conversely, for levels >15 ng/mL, the dose is decreased by 25% until target range is achieved. All dose adjustments will be rounded to the nearest whole number. Levels should also be drawn after changing the dose of sirolimus or adding any of the medications known to interfere with the sirolimus metabolism (**Table 4**).
d) Blood pressure, renal function tests (creatinine, BUN), electrolytes and magnesium need to be followed at least two to three times per week while receiving sirolimus to full dose and then twice weekly or per attending until sirolimus is discontinued
e) Patients who are experiencing either suspected or documented fungal infection, alternative therapy should be administered whenever possible. If voriconazole, posaconazole, or fluconazole are deemed necessary, sirolimus dosing reductions must be followed[86;87].
e) Severe neutropenia or thrombocytopenia: A thorough evaluation cause of marrow suppression should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for grade IV neutropenia and thrombocytopenia that persists and in the case of neutropenia is refractory to G-CSF therapy. Dose reductions of sirolimus of approximately 50% should occur. For severe persistent toxicity despite sirolimus dose reduction, sirolimus should be held until blood counts recover to ANC > 1000/ul and platelets >75,000/ul. At that point, sirolimus may be reintroduced at a 1 mg PO QD and dose increased to goal as long as severe hematopoietic toxicity does not occur

4. Growth Factor Support

Patients will receive G-CSF at 5 μg/kg/day IV or SC starting at day +5 and continuing until the ANC >500/uL for 3 consecutive days. Refer to **Section IX.B.3.c.iii** for information regarding prolonged neutropenia while on MMF.

5. Donor NK Cell Collection, Processing, and Infusion

a. Collection of Donor Mononuclear Cells and Enrichment/Purification of Donor NK Cells

All efforts will be made to collect peripheral blood mononuclear cells (PBMCs) from the donor using apheresis on Day +6. If it is not feasible to collect cells on Day +6, PBMCs can be collected between Days

+6 to +8 to be infused between Days +7 and +9 (the strong preference is that they be collected on Day +6 and infused on Day +7). Once collected, the apheresis product will be transported to the local Cell Processing Lab on the day of collection.

Once the apheresis product has arrived, PBMCs will undergo processing that consists of CD3 depletion followed by CD56 selection using the Miltenyi CliniMACS system using GTP standard procedures. Details regarding the enrichment and purification processing can be found in the CMC document related to the IND for this protocol.

Briefly, the FDA has approved two routes of processing once PBMC arrive to the lab:

1) Overnight PBMC storage at 1-6 C, then sequential CD3 depletion and CD56 selection the following day (1 day processing); OR
2) Begin CD3 depletion on arrival to the Cell Processing Lab, followed by overnight storage of the CD3 depleted product. CD56 selection occurs the next day (2 day processing).

Please refer to the CMC document of the IND (BB # 13794) for further details and explanations regarding options for 1 or 2 day processing. Both options are considered equivalent based on validation results. The preference would be for 1 day processing.

The NK cell product will be stored at room temperature until ready for infusion.

If patients are receiving greater than or equal to 0.25 mg/kg/day equivalent prednisone dose within 1 day of the NK cell infusion, donor NK cells will not be infused on day +7.

If already collected and processed:

- *Donor NK cells may be infused within 3 days after selection (from day +7 to +9) as long as the patient is on <0.25 mg/kg/day equivalent prednisone.*
- *NK cells collected and purified in excess of those required to meet the infusion dose will be maintained for quality control purposes according to the participating lab policies/procedures*

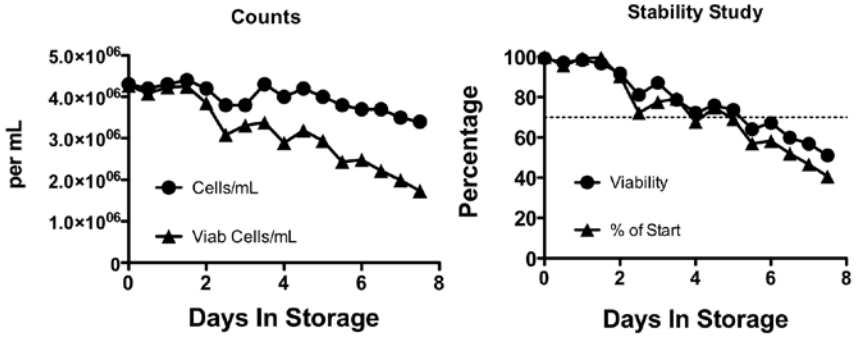


Fig 11. Viability study evaluating NK cells over time shows excellent stability over 3 days suggesting that NK cells should still have appropriate activity within 3 days of manufacturing if maintained at room temperature.

Data from our internal validation studies at MCW demonstrates the ability of NK cells to be viable and stable up to 3 days from collection/processing if left at room temperature (**Fig 11**). In the case that a patient does not receive NK cells for any reason, the patient will remain on study and outcomes will be collected but may not count toward accrual goals based on the specific clinical scenario and per the DSMB's recommendations.

b. Characterization and Release of NK Cell Product

An aliquot of the enriched product will undergo cellular phenotyping per standard operating procedures of the Cell Processing Lab. The number of CD3+ and CD56+ cells from the unmanipulated apheresis product will be determined prior to processing to define the degree of NK cell enrichment and log T cell depletion.

The NK product will be deemed appropriate for release if the following three criteria are met:

1) Immediate sterility assessment (i.e., negative gram stain) is clear of foreign organisms;
2) Cell viability, as ascertained by flow cytometry, is ≥70%; and
3) Number of residual CD3+ cells is $<10^4$/kg.

It may be necessary to reduce the volume of the NK cell product to achieve the appropriate number of CD56+/CD3- cells. Additionally, the volume may need to be reduced to achieve a residual CD3 cell number $<10^4$/kg. Otherwise the product will not undergo any further manipulation to reduce the number of CD3+ cells.

The goal NK cell dose level on this study is 5.0 x 10^6 CD56+/CD3- cells per kg.

An infused NK cell dose as low as 2.0 x 10^6/kg or as high as 8.0 x x 10^6/kg will be accepted as achieving a successful NK cell dose and will be counted toward accrual goals.

Any patient who fails to receive a successful NK cell dose for any reason will remain on study but may not be counted toward the safety and/or efficacy endpoints unless deemed appropriate by the Data Safety Monitoring Board (DSMB).

The enriched NK cell product will be extensively phenotyped for expressed NK cell receptors and any uninfused NK product will be cryopreserved and stored in the Cell Processing Lab. NK byproducts will be phenotyped and stored only as required by the Cell Processing Lab as part of their quality assurance process.

c. Infusion of Donor NK Cells

All efforts will be made to infuse donor NK Cells into the recipient on day +7 as long as three release criteria are met (refer to **IX.B.5.b**). The infusion of these cells will follow established standard procedures for donor lymphocyte infusions, including having anaphylaxis medications immediately available, and having close monitoring during and after the infusion (for up to 2 hours from the end of the infusion). No pre-medications will be given unless clinically necessary (for example, prior history of severe reactions or previous anaphylaxis). Having a prior reaction to blood products unto itself is not a pre-requisite to requiring pre-medications for NK cell infusion.

Adult patients may be given the NK cell product as an outpatient based on clinical status. However, adult patients may need to be admitted to the inpatient service in cases of delayed product arrival or need for post-infusion monitoring beyond the hours of the outpatient clinic. All pediatric patients will receive the NK cell product as inpatients.

Standard guidelines for cellular product infusion currently in place require a 2 hour monitoring period to observe any potential adverse events from the end of infusion. Per the FDA, as this is a Phase II study, only 2 hours post-infusion monitoring is required. Any infusional reactions (grade IV toxicity using the Adapted Common Toxicity Criteria) will be recorded and reported to the FDA in an expedited manner. All infusional observations/toxicities will be recorded on the NK Cell Infusion Worksheet found in **Appendix A.**

Potential rare side effects of the NK cell infusion include anaphylaxis, nausea, and vomiting. Infection, diarrhea, and marrow suppression, in particular prolonged neutropenia, are considered very rare. One extremely rare potential side effect may be the development of human-anti-mouse antibodies (HAMA) from the Miltenyi beads.

Steroids are to be minimized/avoided within 2 days AFTER receiving the NK cell infusion to maximize its effectiveness. If patients must receive steroids due to medical necessity during this time period, this must be documented clearly in clinical notes. Patients will remain on study.

C. Donor Treatment Plan

Table 5. Donor Treatment Plan

	Days				
	Week 4 to 3 Pre-Harvest	**Week 3 to 2 Pre-Harvest**	**Day -1**	**Day 0**	**Day +6**
Autologous RBC Collection (2 Units)[1]	●	●			
Oral iron supplementation x 7 days (for marrow donors only)	●				
PBSC Collection[2] Collection occurring on the 5th (day -1) or 6th (day 0) day of G-CSF			●	(●)	
Marrow Harvest[2]				●	
Infusion of Autologous RBC (1-2 Units)[1]				●	
Leukapheresis for NK collection[3]					●

[1] For marrow donors only: Based on weight discrepancy between patient/donor and requirements of marrow harvest, autologous blood unit collection may be limited to only **one collection**. If only 1 Unit is needed, the collection can take place between **1 to 4 weeks** prior to bone marrow harvest.
[2] Donors will provide **either** fresh marrow (collected on day 0) **or** fresh mobilized PBSC (collected on day -1, with ability to have a second collection on day 0 should there be need). **Marrow is the preferred cell dose over PBSC.** Cryopreserved donor PBSC may be used in place of fresh PBSC in select cases.
[3] Leukapheresisfor NK cell collection may take place between days +6 and +8 to allow for scheduling flexibility. All attempts should be made to perform apheresis on Day +6.

1. Donor Screening Guidelines

Per FDA guidelines, 21 CFR 1271 will be used for screening and testing the suitability of all donors. Donors will be undergoing both marrow harvest or mobilized PBSC collection for the transplant and apheresis for mononuclear cell collection. **Despite being screened for initial donor selection, it is important to note that all donors must be re-screened a minimum of 72 hours before, but no more than 6 days before, apheresis for mononuclear cell collection. This is to remain compliant with FDA regulations 21 CRF 1270 as part of good tissue practice (GTP) guidelines for donor eligibility for cellular product infusions, which mandate donor screening within 7 days of cellular product collection.**

2. Autologous Blood Collection – *This section only applicable for Marrow Donors*

Since donors will undergo COBE apheresis approximately 1 week after marrow harvest, 2 Units of autologous blood may need to be collected from all adult donors as they will become anemic after marrow harvest. For pediatric donors greater than or equal to 12 years of age who meet other eligibility criteria, this amount will be determined based on donor and recipient weights. The first unit will be collected 3-4 weeks prior to the marrow harvest, and the second unit collected 2-3 weeks prior to the harvest. There should be at

least one week between the first and second units. The second unit should be collected no less than 14 days (± 2 days) prior to the marrow harvest. If only one auto collection is needed, this may be collected between 1 and 4 weeks prior to harvest. After each round of RBC collection, it is recommended that the donor be prescribed a 7 day course of oral iron supplementation to help replenish RBC stores.

3. Marrow Harvest and Infusion of Autologous Blood – *This section only applicable for Marrow Donors*

It is assumed that 1 liter of aspirated marrow will translate into the equivalent of at least one unit of blood loss. Additional blood loss occurs from bleeding into soft tissue during and after the procedure. For a harvest of 2 liters, a donor will lose the equivalent of at least 2 units of blood with the product plus an additional 1 to 2 units from bleeding and oozing into the soft tissue (personal communication, M. Linenberger, FHCRC). Platelet loss is not a problem after marrow harvest. The donor marrow harvest will occur on Day 0 per standard procedures. The total nucleated cell (TNC) goal will be based on the recipient's body weight as specified above. Other than standard filtration, in the absence of ABO or other red cell incompatibility, the marrow product will be directly infused into the recipient without manipulation. If major or minor ABO incompatibility exists with significant donor or recipient antibody titers, the product will be manipulated according to standard practice procedures. Following the marrow harvest, donors will be transfused autologous blood to replenish blood loss from the harvest bleeding into tissues, and to minimize anemia prior to planned apheresis at day +6 post-harvest.

4. Mobilized Peripheral Blood Stem Cell Apheresis for PBSC collection– *This section only applicable for PBSC donors*

In certain circumstances, donor marrow collection for transplant may not be achievable (for example, if there is a large weight discrepancy between patient and donor, such that it is not safe or feasible to perform a donor marrow harvest). In such cases, donors will receive 5 or 6 days of filgrastim (G-CSF) subcutaneously to mobilize stem cells. PBSC will be collected via leukapheresis on day -1. Should a second day's collection be required due to low cell dose, a 6^{th} or 7^{th} dose of filgrastim will be given and a second collection can be performed on day 0. The target CD34 dose will be 5.0 x 10^6 CD34 cells/kg of recipient weight. Any extra collected PBSC will be cryopreserved for future clinical use. Donors less than 18 years of age must be able to collect stem cells using peripheral venous access. Adult donors ≥ 18 years of age may be required to undergo temporary central venous access should peripheral access be difficult. This possibility is reviewed during the informed consent process. PBSC will be infused fresh. However, cryopreserved donor PBSC will be an acceptable alternative.

5. Peripheral Blood Mononuclear Cell (PBMC) Apheresis for NK cell collection

Apheresis will utilize either large-bore peripheral IV access, or in adult patients without adequate peripheral veins, a central venous catheter placed on the morning of the apheresis procedure. Donors less than 18 years old must be able to collect cells from a peripheral venous access. Adult donors who also donated PBSC for transplant rather than marrow will be informed that although their PBSC may have been collected using peripheral access, there is a possibility that they many need to undergo temporary central venous access (if peripheral veins have not adequately recovered during the one week interval between PBSC and PBMC collection). This possibility is reviewed in the informed consent process. Mobilization (ie, G-CSF) is not used for PBMC collection from which NK cells will be derived. The apheresis is preferred to take place on day +6 (can occur between days +6 and +8 to facilitate scheduling if necessary). A total of 10 to 12 liters of donor blood volume will be processed by leukapheresis using a Cobe Spectra instrument and following Standard Operating Procedures and standard treatment plans of the Apheresis Unit. The apheresis product

will be transported to the Cell Processing Facility for either overnight storage or to begin processing as described in detail in the Investigator Brochure per IND BB 13794.

X. RECIPIENT AND DONOR EVALUATIONS

A. HLA-Typing of Patient and Potential Donors

1. Even if patients have identified HLA-matched related or unrelated donors, only HLA-haploidentical donors will be considered for this protocol to increase the opportunity for GVT effects. Thus, an Unrelated Donor Search is not required for this protocol.
2. As broad a range of potential HLA-haploidentical related donors as possible should be typed. Included are parents, siblings, eligible children, and cousins.
3. Serotyping (HLA-A, B, C) and DNA typing (HLA-A, B, C, DRB1, DQB1) of patient and donor will be performed.
4. Recipient must have a negative cytotoxic cross-match to donor lymphocytes. Either leukocyte and/or florescence activated cell sorter cross match between the patient and donor or an HLA antibody screen by luminex will be used. If positive, then specific screen against donor HLA antigens must be negative. Otherwise an alternative HLA-haploidentical donor must be identified.
5. KIR genotype will not be used in determining donor selection but data will be collected prospectively for this trial.
6. Of multiple potential HLA-haploidentical donors who could be used, institutional standard care guidelines will be used in donor selection. This could include, but is not limited to, CMV status, ABO type, and donor age and health condition.

B. Recipient Evaluations

1. Pre-transplant Evaluations

Standard of Care:

a) See Institutional Standard Practice Guidelines for Standard Pre-Transplant Evaluation Guidelines. This includes infectious disease testing/imaging and testing for baseline organ function to determine pre-existing co-morbidities. HCT-Comorbidity Index (HCT-CI) will be used to score pre-transplant co-morbidity risks[88]. Refer to **Appendix B** for scoring guidance.

b) See **Table 6** for disease-specific pre-transplant evaluations, which must occur within 3 weeks prior to registration to count toward protocol eligibility. Refer to **Appendix C & D** for Solid Tumor Disease Evaluation Guidelines and definitions to gauge response.

Research:

c) See **Appendix E** for blood and tissue research specimens and HRQoL survey requests.

Table 6: Disease-Specific Evaluations for Disease Pre and Post-HCT
(Abbreviations: CT CAP= CT chest/abdomen/pelvis with IV and oral contrast)

Pre-transplant disease staging to take place ≤3 weeks prior to protocol registration
Post-transplant disease staging to take place within ± 2 weeks of the time-point listed.

Table 6.A: Ewing Sarcoma (EWS), Rhabdomyosarcoma (RMS), Neuroblastoma (NB), Osteosarcoma (OS)

	Pre-HCT	+30	+84	+100	6m	9m	12m	15m	18m	21m	24m	At progression/ Relapse
H&PE, VS, Ht, Wt, BSA	x	x	x	x	x	x	x	x	x	x	x	x
CBC & CMP[1]	x	x	x	x	x	x	x	x	X	X	x	x
MRI or CT primary site(s) of recurrence where there is radiographically detectable disease[2] (Plain film x-ray acceptable if hardware present)	x		x		x	x	x	x	x	x	x	x
CT Chest (EWS, RMS, OS ONLY)	x		x		x	x	x	x	x	x	x	x
CT Chest-Abd-Pelvis (NB ONLY)	x		x		x	x	x	x	x	x	x	x
Bone marrow aspirates and biopsies[3]	x											X
Functional imaging[4]	x				x		x		x		x	x
Tumor markers[5]	x		x		x	x	x	x	x	x	x	x
Chimerism Studies[6]		x		x			x					X

[1] Should include at minimum CBC, differential, electrolytes, BUN, creatinine, ALT, bilirubin, and albumin.
[2] MRI preferred for extremity sarcomas. Use the same imaging modality throughout.
[3] Only required pre-transplant if there is a history of prior marrow disease or concern of new marrow disease on PET scan. If morphologic disease is present in marrow at time of transplant, repeat every 3 months until morphologically negative. Repeat only if CBC shows unexpected changes and/or at relapse of RMS, EWS, and NBL. Can omit if metastatic relapse/rapidly-progressing disease is present.
[4] Obtain functional imaging in the form of MIBG for previously-avid or secreting NBL; bone scan or PET scan for Ewing's & Osteosarcoma; PET scan if previously obtained and avid. Substitute bone scan for previously non-MIBG-avid NBL.
[5] If previously positive: For NBL: Urine HVA and VMA. For CNS germ cell tumors: if positive at diagnosis, check β-HCG, and/or AFP in serum and/or CSF (site per original positive test).
[6] Chimerism studies to include: whole blood, CD3, CD33. Can occur more often as clinically indicated.

Table 6.B. Brain Tumors – Due to diversity of diseases, standard of care follow-up may occur at a different schedule based on clinical management of the patient.

	Pre-HCT	+30	+84	+100	6m	9m	12m	15m	18m	21m	24m	At progression/ Relapse
H&PE, VS, Ht, Wt, BSA	x	x	x	x	x	x	x	x	x	x	x	X
CBC& CMP[1]	x	x	x	x	x	x	x	x	x	x	x	X
CSF cytology[2]	x											x
Brain MRI	x		x		x	x	x	x	x	x	x	X
Spine MRI[3]	x		x		x	x	x		x		x	X
Tumor markers[4]	x		x		x	x	x	x	x	x	x	X
Chimerism Studies[5]		x		x			x					X

[1] Should include at minimum CBC, differential, electrolytes, BUN, creatinine, ALT, bilirubin, and albumin.
[2] All patients will have CSF evaluated at study entry. If positive at study entry: CSF for cytology will be checked every three months until negative and then stop.
[3] If positive at study entry, every three months for the first year, every six months for year two. Required at pre-transplant evaluation for medulloblastoma and ependymoma; for other brain tumors, based on clinical suspicion.
[4] For CNS germ cell tumors: if positive at diagnosis, check baseline β-HCG, and/or AFP in serum and/or CSF (site per original positive test). Will also be needed at time of relapse.
[5] Chimerism studies to include: whole blood, CD3, CD33. Can occur more often as clinically indicated.

2. Post-transplant Evaluations

Standard of Care:

a) See Institutional Standard Practice Guidelines for Standard Post-Transplant Evaluation Guidelines. This includes recommended chimerism testing (whole blood, CD3, CD33) at 1 month, Day +100 and 1 year post transplant, and at relapse. Refer to **Appendix C & D** for Solid Tumor Disease Evaluation Guidelines and definitions to gauge response.

b) **See Table 6** for disease-specific transplant evaluations. Patients will have testing based on **Table 6** at the following recommended time-points post-transplant:

- Every 3 months (± 2 weeks) for the first 2 years post-HCT.
    - **Disease testing may happen more frequently or at a different schedule based on clinical management of the patient.**
- Additional disease testing after year 2 post-HCT will take place per institutional practice

RECIST 'Response Evaluation Criteria in Solid Tumors' (RECIST) from the NCI[89] is the recommended criteria to be used to assess radiographic changes in pediatric patients. Data will be used to calculate PFS. In the case of no measurable disease pre-transplant, absence of new disease (see definition in **Section XV.B**) at each time-point will be documented to determine lack of disease progression.

Response Assessment in Neuro-Oncology' (RANO) criteria (see Appendix D) is the recommended criteria to be used to assess radiographic changes in adult patients with brain tumors. Data will be used to calculate PFS. In the case of no measurable disease pre-transplant, absence of new disease (see definition in Section XV.B) at each time-point will be documented to determine lack of disease progression.

Research:

c) See **Appendix E** for blood and tissue research specimens and survey requests.
   i) Blood tests will be performed to evaluate immune reconstitution
   ii) Tissue specimens will be studied to evaluate infiltrating immune cells
      (a) any initial and post-transplant tumor biopsy and/or resection samples
      (b) any post-transplant GVHD diagnostic samples
   iii) Surveys will be conducted to evaluate HRQoL

C. Donor Evaluations

Standard of Care:

1. See Institutional Standard Practice Guidelines for Standard Pre-Transplant Evaluation Guidelines. This includes infectious disease testing and a pre-transplant baseline reference for determination of donor chimerism post-transplant.

Research:

2. See **Appendix E** for **blood research specimen** requests.
   i) Blood tests will be performed to evaluate donor immune function
   ii) Blood tests will be performed to evaluate for cytotoxicities in the donated NK cell product

XI. DRUGS ANDIRRADIATION ADMINISTRATION – TOXICITIES AND COMPLICATIONS

A. Cyclophosphamide

1. Description

Cyclophosphamide is an alkylating agent which prevents cell division primarily by cross linking DNA strands. Cyclophosphamide is cell cycle non-specific. Cyclophosphamide is not stem cell toxic.

2. Storage and Administration

Cyclophosphamide for injection is commercially available in 2000 mg vials, which are reconstituted with 100 ml sterile water for injection. The concentration of the reconstituted product is 20 mg/ml. The calculated dose will be diluted further in 250-500 ml of Dextrose 5% in water. Each dose will be infused over 1-2 hr (depending on the total volume).

3. Side Effects and Toxicity

Clinical toxicities of cyclophosphamide include alopecia, nausea and vomiting, headache and dizziness, hemorrhagic cystitis, cardiotoxicity, immunosuppression, myelosuppression, pulmonary fibrosis, increased hepatic enzymes and syndrome of appropriate anti-diuretic hormone (SIADH).

B. MESNA

1. Description

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazaphosphorine (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazaphosphorine, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazaphosphorine.

2. Storage and Administration

Mesna is commercially available in 200 mg, 400 mg and 1000 mg vials containing a 100mg/ml solution. Each dose of Mesna will be diluted further in 50 ml of normal saline to be infused over 15 min. Mesna dose will be based on the CY dose being given but can be omitted when the CY dose is less than 1000 mg/m^2 (or 33.3 mg/kg). The total daily dose of Mesna is equal to 100% of the total daily dose of cyclophosphamide.

3. Side Effects and Toxicity

At the doses used for uroprotection Mesna is virtually non-toxic. However, adverse effects, which may be attributable to Mesna, include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue.

C. Fludarabine

1. Description

Fludarabine's active metabolite 2-fluoro-ara-A is an antimetabolite that inhibits DNA primase, DNA polymerase alpha and Ribonucleotide nuclease.

2. Dosage and Administration

Fludarabine monophosphate is commercially available as a 50 mg/vial, which is reconstituted with 2 mL of sterile water, resulting in a 25mg/mL solution. The desired dose is further diluted to concentrations of 0.04-1 mg/mL in normal saline or 5% dextrose (50-100 mL) for injection and will be administered by IV infusion over 30 minutes or longer.

3. Side Effects and Toxicities

The dose of fludarabine used in this protocol is nonmyeloablative; however, it can cause severe immunosuppression particularly in the CD4+ T cell compartment. Immunosuppression increases the risk of infection, which can be life threatening. In addition, clinical toxicities of fludarabine monophosphate include: myelosuppression, primarily lymphopenia and granulocytopenia, alopecia, rash, dermatitis, nausea, vomiting, anorexia, stomatitis, diarrhea, somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness, hepatocellular toxicity with elevation in serum transaminases, neurotoxicity and interstitial pneumonitis. These effects are reversible when the drug is discontinued. Immunosuppression observed with the use of fludarabine increases the risk of infection which can be life-threatening.

D. Total Body Irradiation

TBI given at high doses in conventional transplants may cause nausea, vomiting, diarrhea, temporary hair loss, and painful swelling of the salivary glands for a few days. TBI may destroy normal marrow cells in addition to the cancer cells. The dose of TBI (300 cGy) used in this protocol is about one-sixth of that used in conventional transplant protocols, and severe acute side effects have so far not been observed. TBI has been associated with causing sterility at high doses and there is a risk of major genetic damage to any children conceived after transplantation. There is a risk that a small percentage of patients may develop a secondary cancer resulting from this treatment.

E. Granulocyte-colony stimulating factor (G-CSF)

1. Description

G-CSF (Filgrastim, Neupogen®) is a biosynthetic hematopoietic agent that is made using recombinant DNA technology in cultures of Escherichia coli. G-CSF stimulates production, maturation and activation of neutrophils. In addition, endogenous G-CSF enhances certain functions of mature neutrophils, including phagocytosis, chemotaxis and antibody--dependent cellular cytotoxicity.

2. Dosage and Administration

G-CSF is supplied in vials containing 300 mcg and 480 mcg of G-CSF at a concentration of 300mcg/ml. The intact vials should be stored under refrigeration. The vials can be left out of refrigeration for 24 hours, but should be discarded if left at room temperature for longer periods of time. G-CSF can be drawn up into tuberculin syringes for administration and stored under refrigeration for up to 7 days prior to usage. G-CSF can be further diluted for intravenous infusion in 5% dextrose. Do not dilute in saline--precipitate may form. If the final concentration of this product is <15 mcg/ml, it is recommended that albumin be added to a final concentration of 2mg/ml (0.2%) to minimize adsorption of the drug to infusion containers and equipment.

3. Side Effects and Toxicity

G-CSF causes marked leukocytosis. Adverse reactions reported commonly include bone pain, thrombocytopenia, diarrhea, nausea, rash, alopecia, fever, anorexia and pain or bruising at the injection site. Allergic reactions, MI, atrial fibrillation, and splenomegaly have been reported rarely. G-CSF is contraindicated in research participants with allergy to E. coli derived products.

F. Tacrolimus

1. Description

Tacrolimus, also known as FK-506, is a macrolide immunosuppressive agent. Tacrolimus inhibits lymphocytes by forming a complex with FKBP-12, calcium, and calmodulin, leading to the decrease in the phosphatase activity of calcineurin. Calcineurin mediates the first intracellular signal required for T-cell activation after antigen recognition by the T-cell receptor. This drug is used with corticosteroids for prophylaxis of organ rejection in patients receiving allogeneic liver transplants and for prophylaxis of GVHD in the setting of HCT. It is also used for immunosuppression after kidney, cardiac, pancreas, pancreatic islet cell and small bowel transplantation. This drug is well-absorbed orally. It is metabolized in the liver by unknown mechanisms, but demethylation and hydroxylation have been proposed based on *in vitro* studies. The metabolized products are excreted in the urine.

2. Dosage and Administration

a) Oral

- Tacrolimus capsules – 0.5 mg, 1 mg or 5 mg capsules. (There is currently no liquid solution available.)
- For better absorption, it is recommended that Tacrolimus capsules be taken on an empty stomach.
- Tacrolimus should not be taken with grapefruit juice as it may increase blood levels.
- If patient vomits within one hour of oral administration, repeat dose.
- If vomiting persists, switch to IV administration.

b) Intravenous

- Sterile solution of 5 mg/mL ampules in polyoxyethylated castor oil (Cremophor FCL).
- Diluted in D_5W in glass or other non-PVC container.
- Final dilution volume: 50-250 mL dependent upon patient size and Tacrolimus dose.
- Infusion time:
  - Pediatrics (<30 kg) – 22-24 hours (continuous infusion 0.03 mg/kg/day). Convert to twice daily oral dosing once therapeutic levels achieved.
  - Adults and Pediatrics (>30 kg) – single daily IV dose of 1 mg/kg over 1-2 hours. Convert to twice daily oral dosing once therapeutic levels achieved.

c) Conversion from IV to PO dosing of tacrolimus.
Patients should be converted to an oral dose at 4 times the IV dose to be given in divided (Q 12 hour) doses. For children aged < 6 years old who have sub-therapeutic levels, the dose interval may need to be reduced to every 8 hours.

3. Side effects and Toxicities

- Renal – Rise in serum creatinine, hemolytic uremic syndrome.
- Neurological – Peripheral: paresthesia, tremor. Central: seizures, headache, insomnia, dizziness, depression, confusion, hallucinations, psychosis, myoclonus, neuropathy, agitation.
- Gastrointestinal – Nausea, vomiting, anorexia, constipation, diarrhea
- Cardiovascular – Hypertension, myocardial hypertrophy
- Endocrine – Hyperglycemia, hyper/hypokalemia, hypophosphatemia, hypomagnesemia
- Integument – Itching, rash.
- Hematologic – Leukocytosis, thrombocytopenia, leukopenia, anemia, PTLD, thrombotic microangiopathy.
- Liver – Abnormal liver function tests.
- Ocular – Blurred vision, photophobia.
- Respiratory – Pleural effusion, atelectasis, cough, dyspnea.
- Musculoskeletal – Arthralgia.

See **Section IX.B.3.b** for information about administration and dosage adjustments.

G. Mycophenolate Mofetil

1. Description

MMF is the morpholinylethylester of mycophenolic acid (MPA) and reversibly inhibits inosine monophosphate dehydrogenase, particularly the type II isoform that is more prominent in activated lymphocytes. As a result of the inhibition of de novo purine synthesis, proliferation of B and T lymphocytes is blocked and antibody production is inhibited.

2. Storage and Administration

MMF is available in an oral and an intravenous formulation. The oral formulation is supplied in 250mg hard gelatin capsules and can be stored at room temperature. MMF for IV administration is supplied as a lyophilized powder in a glass vial containing the equivalent of 500mg.

3. Side Effects and Toxicity

MMF has been studied extensively among patients after nonmyeloablative HCT. Previous clinical studies in patients after allografting suggest that the principal adverse reactions associated with the administration of MMF include nausea, vomiting, neutropenia, diarrhea, and on one occasion bloody diarrhea. In the setting of HCT, several etiologic factors may contribute to alterations in gastrointestinal and hematologic parameters. MMF has an increased incidence of digestive system adverse events, including GI tract ulceration, and hemorrhage (3% of patients receiving MMF). GI tract perforations have rarely been observed. Most patients in these studies were also on other drugs known to be associated with these complications. Up to 2% of patients receiving MMF for prevention of rejection developed severe neutropenia (ANC <500). The development of neutropenia may be related to MMF itself, concomitant medications, viral infections or some combination of these causes. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the principal investigator and documented in the medical records and the clinical reporting form (CRF). Dose adjustments are described in Section **IX.B.3.c**.

H. Sirolimus

1. Description

Sirolimus inhibits the response to interleukin-2 (IL-2), and thereby blocks activation of T and B cells. The mode of action of sirolimus is to bind the cytosolic protein FK-binding protein 12 (FKBP12) similar to tacrolimus. However, the tacrolimus-FKBP12 complex inhibits calcineurin, while the sirolimus-FKBP12 complex inhibits mTOR by directly binding to the complex. Metabolism of sirolimus is by the intestinal and hepatic CYP3A family of enzymes and likely contributes to variability in absorption. It is highly protein-bound.

2. Storage and Administration

Sirolimus is supplied as oral solution (Rapamune Oral Solution) 1 mg/mL or as tablets (0.5 mg, tan; 1 mg, white; 2 mg, yellow-to-beige).

Rapamune Oral Solution pouches should be stored protected from light and refrigerated at 2°C to 8°C. If necessary, the patient may store the pouches at room temperature up to 25°C (77°F)

for a short period of time (e.g., several days, but no longer than 30 days). The tablets should be stored at 20-25°C and be protected from light.

Sirolimus is to be administered orally once daily as it has a long half-life (~60-70 hours) although it may be considerably shorter in children and may require twice-daily dosing. To minimize variability of exposure to sirolimus, this drug should be taken consistently with or without food. Grapefruit juice reduces CYP3A4-mediated metabolism of sirolimus and should not be administered with sirolimus or used for dilution.

If patients are receiving Rapamune Oral Solution, the dose should be mixed well with 60 mL of water or orange juice and taken immediately. It is recommended that the container be refilled with a minimum of 120 mL of water or orange juice, mixed well, and this rinse solution should be swallowed.

3. Side Effects and Toxicity

- Liver - Hypercholesterolemia, hyperlipidemia, diarrhea,
- Cardiac – Hypertension
- Integument – Rash, arthralgias
- Hematologic – Anemia, neutropenia, thrombocytopenia
- Renal – Hypokalemia, hemolytic uremic syndrome
- Neurological - Seizures

See **Section IX.B.3.d** for information about administration and dosage adjustments.

XII. INFECTIOUS DISEASE PROPHYLAXIS AND THERAPY

Patients will receive prophylaxis for pneumocystis carinii pneumonia (PCP), varicella zoster virus (VZV), herpes simplex virus (HSV), and encapsulated bacterial and fungal organisms per Institutional Standard Practice Guidelines.

Highlights include, but are not limited to:

1. Sulfamethoxazole/trimethoprim should be started pre-transplant, discontinued on day -1, and reinstituted at Day +30 only if the ANC >500 for three consecutive days; continue until off all immunosuppression. Use alternative prophylaxis if ANC <500 by Day+30.
2. Acyclovir should be provided to all patients except those having both negative HSV and VZV serostatus and continued until off immunosuppression.
3. Fluconazole should be started pre-HCT and continued until off all immune suppression.

XIII. GRAFT-VERSUS-HOST DISEASE

A. Diagnosis and Treatment

1. Refer to **Appendices E and F** for diagnosis and Institutional Standard Practice Guidelines for latest treatment recommendations for acute and chronic GVHD, respectively.
2. Prophylaxis with daily Bactrim, acyclovir, and fluconazole (or equivalent antimicrobials) must continue while receiving immunosuppression for Acute GVHD >Grade II or Chronic Extensive GVHD

B. Stopping Rules for GVHD

A stopping rule is provided that will stop the study if there is evidence that the incidence of grade III/IV acute GVHD is greater than 30% within the first 100 days after HCT. Details of the rule are described in **Section XV.**

XIV. EVALUATION AND ENDPOINT DEFINITIONS

A. Study Description

This is a Phase II study using nonmyeloablative conditioning (FLU, CY, TBI), HLA-haploidentical HCT, potent post-transplantation immunosuppression (CY, MMF, tacrolimus), followed by a single NK cell infusion early after HCT for pediatric and adult patients with high-risk non-hematopoietic sold tumors.

B. Definition of endpoints

- Refer to **Appendix C & D** for definitions and guidance on evaluating measurable disease

1. Progression: Advancement of malignancy after HCT despite previous achievement of stable disease. Disease may be evaluated by imaging and/or biopsy (when indicated).

2. Relapse: Recurrence of malignancy after having prior evidence of remission. Disease may be evaluated by imaging and/or biopsy (when indicated).

3. Progression-free survival (PFS): The length of time after transplant that the patient lives with the disease but the disease does not get worse

4. Disease Control Rate: The percentage of all patients who have achieved complete response (CR), partial response (PR), and stable disease (SD) to a therapeutic intervention in clinical trials of anticancer agents. Equals to CR + PR + SD.
5. Overall survival (OS): The length of time after transplant that the patient lives. Disease status is not part of the definition.

6. Non-relapse Mortality (NRM): Death in any patient for whom there has not been a diagnosis of relapse or disease progression.

7. Acute GVHD:
   a. Definition: Acute GVHD will be defined by the criteria outlined in **Appendix F.**
   b. Evaluation: GVHD will be assessed by a by a single clinician using the criteria in **Appendix F.** Biopsy for confirmation of diagnosis of skin, liver and GI tract GVHD will be performed as indicated.
   c. The time to onset of GVHD and the number and types of immune suppressant therapies will be determined.

8. Chronic GVHD:
   a. Definition: Chronic GVHD is defined by criteria outlined in **Appendix G.**
   b. Evaluation: Chronic GVHD endpoints will include:
      i. Extent (limited or extensive)
      ii. Duration of immune suppression in months after diagnosis
      iii. Number of cycles of immune suppressant therapy
      iv. Need for immune suppressive agents other than first line therapy

9. Donor engraftment definitions
   a. Engraftment: Time to absolute neutrophil count (ANC) >500/uL on the first of three consecutive days.

   b. Mixed chimerism: 5-95% donor T-cells (CD3+) in peripheral blood.

   c. Full donor chimerism: > 95% donor CD3+ T-cells in peripheral blood.

   d. Donor engraftment: Having mixed or full donor chimerism.

   e. Increasing donor chimerism: a 20% absolute increase in the CD3+ T-cell chimerism compared to the previous months' chimerism evaluation.

   f. Decreasing donor chimerism: a 20% absolute decrease in the CD3+ T-cell chimerism compared to the previous months' chimerism evaluation.

   g. Low donor chimerism: <40% CD3+ T-cells after HCT on two consecutive evaluations within a 4 week period. The two evaluations must be at least 14 days apart. Low donor chimerism should always be confirmed with repeat blood T-cell and granulocyte chimerism analysis. VNTR analyses or FISH studies (in sex mismatched patients) of sorted peripheral blood CD3+ T-cells will be used to quantify chimerism. The same assay should be used for a given patient for repeated studies of chimerism. VNTR and FISH analyses will also be performed on marrow aspirates. Therapeutic decisions (i.e. donor lymphocyte infusion) will be made based on the results of sorted T-cell studies of peripheral blood.

   h. Graft rejection: the inability to detect, or less than 5% donor T-cells (CD3) as a proportion of the total T-cell population after nonmyeloablative HCT.

   i. Graft failure: grade IV thrombocytopenia and neutropenia after Day +21 that lasts > 2 weeks and is refractory to growth factor support. Primary graft failure occurs when no preceding engraftment occurs. Secondary graft failure occurs when preceding engraftment is seen.

XV. STATISTICAL ANALYSIS

Dose-limiting toxicity (DLT) is defined as having at least one of the following adverse events, *independent of the attribution to the NK cell infusion,* within the first 28 days after the NK cell infusion (ie, within day +35 after transplant assuming NK cells are infused on day +7) **and will require expedited reporting to the FDA**:

1) Grade IV infusional toxicity (based on the "Adapted Common Toxicity Criteria" or CTC 4.0- **Appendix H)**
2) Grade IV regimen-related toxicity (based on Adapted CTC 4.0 – **Appendix H**)
3) Grade IV acute GVHD
4) Non-relapse mortality

Because of the preliminary favorable safety profile obtained in Protocol 2230 (where no DLTs as defined above have occurred to date and which utilizes a similar conditioning, immunosuppressive, and NK cell dose regimen as described in the current study), this protocol is developed as a free-standing Phase II trial. Safety evaluations will continue to occur as part of the endpoints of this study. Particular emphasis to safety will be observed within the first 100 days after transplant, with the first 35 days after transplant a particularly

cautious period due to the slight augmentation of the treatment regimen (adding 1 extra gray of TBI on day 0 and one extra dose of post-transplant CY on day +4).

Because of the heterogeneity of the patient populations, relapse rates are not likely to be directly comparable, and further analysis taking this heterogeneity into account will be required in order to determine the potential effectiveness of the regimen. While patients with localized solid tumors generally fare well, patients with relapsed and refractory disease have a very poor prognosis when treated with additional conventional therapies. Overall survival in this high-risk population is less than 20% with most patients experiencing further progression of their disease within six months. New therapeutic regimens that utilize novel anticancer treatments merit study in this patient population.

This protocol will target a total enrollment of 20 patients. With 20 patients we can estimate the disease control rate with a standard error of 11%.

To ensure that the treatment regimen is not too toxic and that safety is preserved, we will examine the following early stopping rules. Each stopping rule will be based on the number of cases with the condition among the first 6, 12 or 18 cases. All four stopping rules are based on an exact binomial stopping rule based on a 5% level test of the hypothesis that the true event rate is as indicated against the hypothesis that it is that large or larger. See **Table 7** for details of the stopping probabilities. Should a stopping rule be met, the protocol will be placed on hold and reviewed by the DSMB to determine potential study modifications (for example, if disease eligibility should be restricted).

Rule 1. Transplant-related mortality ≥ 25% by day +100
Stop if we observe 4/6, 8/12 or 9/18 cases that die from the treatment prior to day +100. The stopping rule requires 6, 12 or 18 cases with 100 days of follow-up and stops with a probability of 0.05 or more when the true transplant related mortality is 25% or greater.

Rule 2. Severe toxicity ≥ 20% by day +35
Stop if we observe 5/6, 6/12 or 7/18 cases of Grade IV toxicity based on Adapted Common Toxicity criteria 4.0 by day +35. This rule stops the trial with a probability of 0.05 or more when Grade IV Adapted Common Toxicity criteria 4.0 is 20% or greater.

In general, many post-transplant toxicities are severe and are routinely expected. These can include, but are not limited to, nausea, vomiting, diarrhea, cytopenias, pain, and infection. Only those very severe, life-threatening Grade IV toxicities listed per the Adapted CTC 4.0 in Appendix H within the first 35 days after transplant will be considered for this stopping rule.

Rule 3. Primary graft failure ≥ 20% by day +100
Stop if we observe 5/6, 6/12 or 7/18 cases who fail to engraft by day 100 (graft failure). This rule stops the trial with a probability of 0.05 or more when the graft failure rate is 20% or greater.

Rule 4. Grade III/IV acute GVHD ≥ 30% by day +100
Stop if we observe 5/6, 7/12 or 10/18 cases develop Grade III/IV acute GVHD by day +100. This rule stops the trial with a probability of 0.05 or more when the Grade III/IV acute GVHD rate is 30% or greater.

These stopping rules will be evaluated at least every 6 patients. Both the patients and events observed among the 6 patients enrolled will 'count' for purposes of evaluating the stopping rules. These rules are based on an exact binomial test of the hypothesis that the indicated rate is larger than the predicted rate. Enrollment may continue pending evaluation of these endpoints at the 6 patient benchmarks.

Table 7. Probability of rejecting null hypothesis and stopping trial

True Event rate	20% Event rate Stop if 5/6, 6/12,7/18 (Rules 2 & 3)	25% Event rate Stop if 4/6, 8/12, 9/18 (Rule 4)	30% Event rate Stop if 5/6,7/12,10/18 (Rule 1)
0.15	1.3%	0.6%	0.1%
0.20	5.5%	2.0%	0.5%
0.25	14.7%	5.0%	1.9%
0.30	28.8%	10.8%	5.0%
0.35	46.1%	20.2%	11.1%
0.40	63.4%	33.3%	20.7%
0.45	78.0%	48.8%	33.9%
0.50	88.4%	64.5%	49.5%

Endpoints to be evaluated include disease control rate, engraftment, overall survival, progression-free survival, response rates, non-relapse mortality, acute GVHD, and chronic GVHD. The primary endpoint, disease control rate (CR + PR + SD) at 6 months, will be estimated using proportions, and confidence intervals will be constructed using Clopper-Pearson intervals. Response rates (CR+PR) will be described using proportions and confidence intervals estimated using Clopper-Pearson intervals. Overall survival will be assessed by the Kaplan-Meier estimator and the other endpoints by cumulative incidence curves with death as a competing risk. Disease progression will be assessed at 3, 6, and 12 months post-transplant and will be summarized for different disease categories. Progression-free survival will be assessed using the Kaplan-Meier estimator.

Immune reconstitution will be analyzed using descriptive statistics. This includes, but is not limited to, changes in individual immune function from baseline compared to post-HCT time points.

HRQoL will be analyzed using descriptive statistics. Mean summary scores for each of the dimensions (physical, emotional, social well-being) will be tabulated. These scores will be reported on a 0-100 scale in which higher scores connote better functioning. For the HSCT module, the scores are reversed, such that higher scores connote greater impact (more hassles, greater distress, worse body image). Data for this study will be compared to average scores in the Tufts databank regarding transplant recipients and patients with solid tumors who have not undergoing HCT.

XVI. DATA SAFETY AND MONITORING PLAN AND ADVERSE EVENT AND SAFETY MONITORING

A. Monitoring the progress of trials and the safety of participants

- *The monitoring and reporting of adverse and serious adverse events on this protocol will follow 21CFR 312.32*
- *This protocol complies with 21 CFR 50, Subpart D, which restricts allowable risk exposure for research not offering the prospect of direct benefit in the pediatric population. This study offers the prospect of direct clinical benefit for children.*

The Principal Investigator (PI) and the Children's Hospital of Wisconsin Pediatric Hematology/ Oncology/BMT Clinical Trials Office (CTO) will supervise this trial as the coordinating site for this study. Oversight will be provided by a dedicated Data Safety and Monitoring Board (DSMB) provided through the MCW Cancer Center, the Children's Hospital of Wisconsin Institutional Review Board (CHW IRB), the Food and Drug Administration (FDA), and an external Monitoring group who will ensure all regulatory aspects of the study are in order. The IND for this protocol is cross-referenced to BB IND #13794 (Holder/Sponsor: M.Thakar)

The PI reviews toxicity data weekly for the first 100 days after HCT, and outcome data for each individual patient at a minimum of 3 months after HCT. Severe adverse events (SAEs) occurring at all sites are reported to the coordinating site, the study nurse, or directly to the PI within 48 hours of recognition of the event. An official report of a severe adverse event is formally created by the (CTO) within five days. The SAE is reviewed by PI. If the SAE meets CHW IRB criteria for expedited reporting, then an official signed report is submitted to the IRB within 5 days. Adverse events which did not meet the CHW Prompt Reporting Criteria as outlined in the CHW IRB policy and procedure should be reported to the IRB at time of continuing review. All deaths, regardless of the cause, need to be reported to the PI within 48 hours and then reported to the IRB.

Patient safety on this clinical trial will be monitored by the MCW Cancer Center's Data Safety Monitoring Board (DSMB). The DSMB will convene prior to study initiation and a minimum of twice a year (unless an ad hoc meeting is requested) to review all outcome data including all dose limiting toxicities and adverse events reported to the IRB. The DSMB will confirm that the trial has not met any stopping rules and will review any patient safety problems necessitating discontinuation of the trial. A summary of findings reported from the DSMB is submitted to the IRB as well as to the FDA as part of the annual review. As with initial review, annual IRB review and approval is also required. **Appendix L** defines the specific roles and responsibilities of the DSMB.

A summary of the MCWCC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety.
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 & 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge.)
- Review all DSM reports.
- Submit a summary of any recommendations related to study conduct.
- Terminate the study if deemed unsafe for patients.

A copy of the MCWCC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary. Any available DSMC letters will be submitted to the IRB of record as required.

This protocol will be monitored by an individual or group of individuals who is not directly or indirectly responsible for the supervision of this trial at a minimum of once per year to ensure all regulatory aspects of this study are in compliance with the FDA. The IND associated with this protocol (BB #13794) requires external monitoring of this trial. The monitoring report, including any corrective action, will be supplied to the FDA as part of the IND annual review.

With respect to safety, patients are monitored for the development of GVHD, infections, dropping donor chimerism, and rejection. All patients, regardless of diagnosis, will be considered in the safety analysis if conditioning has started. GVHD events will be closely monitored and severity of GVHD graded. Formal stopping rules are in place for severe toxicity before day +35, and GVHD grades III-IV, graft failure, and TRM before day +100.

Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic, or referring clinicians at other institutions, and is transmitted to the Trial Coordinator and CTO. As with all academic institutions, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes in the medical record. Thus, multiple health

care providers provide independent observations and participate in monitoring patient safety in this trial. The PI may be a clinician for some patients entered on this trial. However, assessments are the sum total of the primary health care provider (resident, fellow, nurse practitioner, physician assistant), floor or outpatient nurse, or another attending clinician involved with the patient, avoiding possible conflict of interest having the PI as the attending clinician for protocol patients. If determination of adverse events is controversial, co-investigators will convene with the PI on an ad hoc basis as necessary to review the primary data and render a decision.

Start-up meeting:
Once other participating institutions are identified and have received IRB approval, the overall PI will hold a start-up teleconference with the participating sites prior to patient enrollment. The site PI and site study coordinator will be required to attend one of these teleconferences. The overall PI will review the overall treatment plan, informed consent procedure, adverse event reporting guidelines, regulatory requirements and requirements for data and sample collection and transmission. A summary of the start-up meeting will be reviewed and signed by the overall PI and the site PI and a copy will be kept in the coordinating center regulatory binder and the site regulatory binder.

B. Plans for assuring compliance with requirements regarding the reporting of adverse events

The descriptions and grading scales found in the Adapted NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 in **Appendix H** will be utilized for adverse event (AE) reporting.

The following definitions will be used to define AEs for this protocol:

Adverse event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Life-threatening adverse event or *life-threatening suspected adverse reaction*
An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or *serious suspected adverse reaction*
An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Suspected adverse reaction
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. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or *unexpected suspected 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, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, 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.

Note:
Re-Hospitalization of the transplant patients, in general, will not be considered a serious adverse event as approximately 75% of evaluable patients are hospitalized. Hospitalization will be considered a serious adverse event if it fulfills the criteria for a serious and unexpected adverse event as described above.

The adverse event reporting will follow guidelines as listed in **Appendix I.** These guidelines detail the expedited reporting requirements and definitions of particular events. Collaborating institutions will report **all unexpected AEs and SAEs that are at least possibly related to the NK infusion** to the PI **within 48 hours of occurrence** using their local IRB guidelines and report forms. All other SAEs are reported to the coordinating site within 5 days by the investigator, trial coordinator, or research nurse upon learning of the event. A completed SAE report form, signed by the PI, must be received by the IRB within 5 calendar days if the SAE meets the following criteria: unexpected; possibly, probably, or definitely related to the research; life-threatening; or results in death. Health care providers communicate with the PI, trial coordinator, or research nurses as events occur triggering subsequent reporting. All serious adverse events are reported to the IRB per guidelines. Adverse events that which did not meet the CHW Prompt Reporting Criteria as outlined in the IRB policy and procedure should be reported to the IRB with a study's continuing review forms. The PI for a study is responsible for this reporting and the CTO assures adverse event reporting on an annual basis. For any applicable grants, the PI will summarize reports of toxicities. Furthermore, stopping rules and interim analysis provides an additional safeguard for adverse event analysis and reporting in this protocol. All collaborating PIs have fulfilled all NIH requirements for training in human subjects' protection.

In regards to monitoring for the FDA, all acute adverse event monitoring will occur until 28 days after the NK cell infusion (35 days after transplant assuming NK cells are infused on day +7). We will plan to report all unexpected SAEs and grades 3-4 infusional reactions in an expedited fashion. We plan to expedite reporting on any patient with an ANC < 500 by day 28 after NK cell infusion. In addition, we will plan to include a separate listing of infusional toxicities, in addition to other required elements, in our annual reports to the FDA.

Duration for monitoring GVHD, relapse, and survival *for purposes of reporting to the FDA* will be for **1 year after transplant.** We will continue collecting long-term follow-up data for many years for our internal use.

C. Plans for assuring that any action resulting in a temporary or permanent suspension of a funded clinical trial is reported to the grant program director responsible for the grant

The procedure of CD3+ depletion and CD56+ selection will be performed under an investigator-sponsored IND, with FDA oversight. Serious and unexpected adverse experiences must be reported to the Principal

Investigator. The principal investigator must report all serious and unexpected adverse events to the IRB, the FDA, and Miltenyi Biotec, Inc. Any temporary or permanent suspension, as determined by the PI, IRB, or DSMB, of this clinical research trial will be reported to the any grant program director by the PI

1. Unexpected and related fatal or life-threatening events are reported to FDA by phone or FAX within 7 calendar days.
2. A written report will be sent to FDA within 15 calendar days for any serious unexpected adverse events for which is a reasonable possibility that the event may have been caused by study device.
3. All adverse events occurring during this study, whether or not attributed to study drug, will be included in the investigator's annual IND report to FDA.

D. Plans for assuring data accuracy and protocol compliance

Refer to the Study Coordinator's Manual (**Appendix I**) and Coordinating Center Functions (**Appendix J**) for details regarding requirement for protocol compliance and reporting.

This protocol has a DSMB that is responsible for reviewing protocol data and safety endpoints. The DSMB meets a minimum of twice a year, or more frequently on an ad hoc basis, to review appropriate endpoint data, and compiles a summary report of findings that is submitted to the PI, IRB and FDA as mentioned above. Refer to the DSMB Monitoring Plan in section **XVI.A and Appendix L** for additional details.

This study will report clinical data using The Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database utilizing study specific electronic case report forms (e-CRFs). Key study personnel are trained on the use of OnCore™ and will comply with protocol specific instructions embedded within the OnCore™ forms. Patient demographics, patient specific study treatment calendars, AEs, reporting of deaths, and other information required for annual reporting will be placed in OnCore™ and other research databases maintained by MCW IT.

Once data has been successfully entered into OnCore™, the principal investigator and his/her staff can extract this data for different analysis software programs. Once an institution consents a patient, they will fax or email the patient demographics and eligibility paper CRF's to the coordinating center which will assign a study ID. Subsequent data will be entered via OnCore™ for review by the coordinating site in the same manner. The study coordinator will review the data reports with Dr. Margolis within 24-72 hours. The overall study coordinator will follow-up with the collaborating institution if there are missing or delinquent data forms, and request submission within a timely manner.

Health care providers and rotating attending physicians assess patients and record their observations in the hospital medical record system. This source documentation is extracted by one of the study nurses or coordinators via chart review and entered into the OnCore™ electronic Case Report Form (eCRF). Patients should be followed as per the required time-points as outlined in the protocol. The study coordinators and/or nurses also continue to follow patients after day 100, review source documentation, and complete eCRFs at 6 months and then yearly intervals per protocol. Protocol Monitoring will be required at all collaborating sites to verify source documentation against entered data completed on eCRF.

XVII. RECORDS

Clinical records will be maintained as confidentially as possible. Data will be collected by study staff and maintained by the OnCore™ database system. The PI will ensure that data collected conform to all established guidelines for coding, collection, key-entry, and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Any publication or presentation will refer to patients by this number and not by name. The licensed medical records department, affiliated with the institution where the

patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are kept in a locked room. Access is restricted to authorized individuals only. Information gathered from this study regarding patient outcomes and adverse events will be made available to the FDA and to Miltenyi Biotec, Inc. All precautions to maintain confidentiality of medical records will be taken.

Any paper data (i.e safety reports, reports generated from EDC) will be kept in a locked and secure location within the Clinical Trials Office of the Pediatric Hematology/Oncology and Bone Marrow Transplant Program. The Clinical Trials Office has secure, electronic employee badge access only. Any paper data that is generated will be kept for at least 10 years.

XVIII. PROJECTED TARGET ACCRUAL

Twenty pediatric and adult patients with rare, high-risk solid tumors are eligible to be enrolled on this multi-site trial over 4 years, with the majority of patients being pediatric. Because of the heterogeneity of the patient population, the following is a projected breakdown of accrual based on the top four high-risk solid tumors treated at the Children's Hospital of Wisconsin from 2010-2012: Neuroblastoma, Wilms Tumor, Rhabdomyosarcoma, and Osteosarcoma. (**Table 8**).

Table 8: Projected and Targeted Accrual

ETHNIC CATEGORY	**Sex / Gender**		
	Females	Males	Total
Hispanic or Latino	1	1	2
Not Hispanic or Latino	8	10	18
Ethnic Category Total of All Subjects	9	11	20
RACIAL CATEGORIES			
American Indian / Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	2	1	3
White	5	9	14
Other	1	2	3
Racial Categories: Total of All Subjects	8	12	20

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APPENDIX A
NK CELL INFUSION WORKSHEET

DATE OF INFUSION:_____ UPN: _____

Prior to Infusion

Patient's weight_____kg

Vital Signs immediately prior to infusion

Time Point	Time	Heart Rate	Blood Pressure	Temperature (°C)	Respirations
Prior to Infusion	____:____ 24-hour Clock				

During Infusion

START TIME OF INFUSION_____

Time Point	Time	Heart Rate	Blood Pressure	Temperature (°C)	Respirations
15 min after start of Infusion	____:____ 24-hour Clock				
30 min after start of Infusion	____:____ 24-hour Clock				
At end of Infusion	____:____ 24-hour Clock				

Adverse Reaction During NK infusion: ☐ None ☐Yes (complete rest of form)

☐ Anaphylaxis reaction requiring medication
☐ Blood pressure instability (hyper or hypotension) requiring medication
☐ Chest tightness/pain
☐ Gross Hematuria
☐ Fever (>38.0°)
☐ Hives requiring medication
☐ Vomiting
☐ Rigors
☐ Cardiac compromise (Brady or Tachycardia) requiring intervention

Post Infusion

STOP TIME OF INFUSION_____

	Time Point	Time	Heart Rate	Blood Pressure	Temperature (°C)	Respirations
	1 hour after Infusion COMPLETED	____:____ 24-hour Clock				
	2 hours after Infusion COMPLETED	____:____ 24-hour Clock				

Adverse Reaction through 2 hrs after completion of NK Infusion:
☐ **None** ☐**Yes (complete rest of form)**

☐ **Physician Notified of Reaction**

☐ **Anaphylaxis reaction requiring medication**
☐ **Blood pressure instability (hyper or hypotension) requiring medication**
☐ **Chest tightness/pain**
☐ **Gross Hematuria**
☐ **Fever (>38.0°)**
☐ **Hives requiring medication**
☐ **Vomiting**
☐ **Rigors**
☐ **Cardiac compromise (Brady or Tachycardia) requiring intervention**

Completed By: _____ Date:_____

PI Signature:_____ Date:_____

RN Signature:_____ Date:_____

☐ **This data is based off of source documentation. (Please check as applicable)**

Appendix B
The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI)

Patient _____ *(name), UPN*_____ *Date*_____

Instructions: Circle applicable scores and provide actual value or cause of co-morbidity. Fax to CHW w/registration

Comorbidities	Definitions	HCT-CI scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventricular arrhythmias requiring treatment in the patient's past history	1	
Cardiac	Coronary artery disease†, congestive heart failure, myocardial infarction in patient's past history or EF of ≤50% at time of HCT	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis requiring treatment in the patient's past history	1	
Diabetes	Requiring treatment with insulin or oral hypoglycemic, but not diet alone, at time of HCT	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular accident in patient's past history	1	
Psychiatric disturbance	Depression/anxiety requiring psychiatric consult or treatment at time of HCT	1	
Hepatic – mild	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN at time of HCT	1	
Obesity	Patients with a BMI of >35 for adults or with BMI-for-age percentile of ≥ 95th percentile for children at time of HCT	1	
Infection	Documented infection or fever of unknown etiology requiring anti-microbial treatment before, during and after the start of conditioning regimen	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica in patient's past history	2	
Peptic ulcer	Requiring treatment in patient's past history	2	
Renal	Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation at time of HCT	2	
Moderate pulmonary	DLco and/or FEV1 >65%-80% or Dyspnea on slight activity at time of HCT	2	
Prior solid tumor	Treated at any time point in the patient's past history, excluding non-melanoma skin cancer	3	
Heart valve disease	At time of HCT excluding mitral valve prolapse	3	
Severe pulmonary	DLco and/or FEV1 ≤65% or Dyspnea at rest or requiring oxygen at time of HCT	3	
Moderate/severe hepatic	Liver cirrhosis, Bilirubin >1.5 X ULN, or AST/ALT >2.5XULN at time of HCT	3	
Please provide (KPS):	Karnofsky Performance Score = _____%	Total Score =_____	Signature of Provider: _____

APPENDIX C
DISEASE EVALUATION CRITERIA AND GUIDELINES – ALL SOLID TUMORS
(With Exception of Adult Brain Tumors)

I. Recommended Response Criteria for All Patients with Solid Tumors

Response and progression will be evaluated in this study using the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Key points are that 5 target lesions are identified and that changes in the *largest* diameter (uni-dimensional measurement) of the tumor lesions but the *shortest* diameter of malignant lymph nodes are used in the RECIST v 1.1 criteria.

II. Definitions

A. Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Radiographically pathologic lymph nodes must be >/= 15mm in short axis to be defined as measurable lesions. Previously-radiated lesions may be included as measurable disease but should be specifically noted as previously-radiated.

B. Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, and inflammatory breast disease, are considered as non-measurable.
Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

C. Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion that can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

D. Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

III. Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. For interpretation of measurable disease, see **Section IV**.

A. Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

B. Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans.

C. PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

D. Tumor markers: Defined as either within the normal range for age ("normal") or elevated ("abnormal"). If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

E. Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain). Cytology should be obtained if an effusion appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease.

F. FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up:
- Is a sign of progressive disease (PD) based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up:
- If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
- If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).

- If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Note: A 'positive' FDG-PET scan lesion means one that is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

G. MIBG Positive Lesions: Patients who have a positive MIBG scan at the start of therapy will be evaluable for MIBG response. The use of 123I for MIBG imaging is recommended for all scans. If the patient has only one MIBG positive lesion and that lesion was radiated, a biopsy must be done at least 28 days after radiation was completed and must show viable neuroblastoma to be included for assessment.

The following criteria will be used to report MIBG response by the treating institution:

- Complete response: Complete resolution of all MIBG positive lesions
- Partial Response: Resolution of at least one MIBG positive lesion, with persistence of other MIBG positive lesions
- Stable disease: No change in MIBG scan in number of positive lesions
- Progressive disease: Development of new MIBG positive lesions

H. Bone Scan Positive Lesions: If performed, bone scintigraphy will also be considered for disease response. Definitions are as follows:

- Complete response: complete resolution of or improvement in all positive lesions
- Partial response: resolution of or improvement in at least one positive lesion, with no new lesions
- Stable disease: no change in number of positive lesions
- Progressive disease: development of more than one new lesion in the absence of known trauma, or development of any new lesion which can be confirmed by another imaging modality
- Non-assessable scan: development of a single new lesion which cannot be confirmed by another imaging modality, or development of multiple new lesions in the setting of known trauma

I. Bone Marrow:

- Complete Response: No tumor cells detectable by routine morphology on bilateral bone marrow aspirates and biopsies.
- Progressive Disease: For patients without marrow disease, detection of tumor in the marrow by routine morphology. For patients with marrow disease at study entry, doubling in the amount of tumor in the marrow AND a minimum of 25% tumor in bone marrow by morphology. (For example, a patient entering with 5% tumor in marrow by morphology must increase to ≥ 25% tumor to have progressive disease; a patient entering with 30% tumor must increase to > 60%).
- Stable Disease: Persistence of tumor in bone marrow that does not meet the criteria for either complete response or progressive disease.

IV. Response Criteria for Patients with Solid Tumors and Measurable Disease

A. Evaluation of Target Lesions

- Complete Response (CR): Disappearance of all target and non-target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. If immunocytology is available, no disease must be detected by that methodology. Normalization of urinary catecholamines or other tumor markers if elevated at study enrollment.
- Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
- Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute

increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions). Note: in presence of SD or PR in target disease but unequivocal progression in non-target or non-measurable disease, the patient has PD if there is an overall level of substantial worsening in non-target disease such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

B. Evaluation of Non-Target Lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)
- Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
- Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

C. Overall Best Response Assessment

Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. The overall response assessment takes into account response in both target and non-target lesions, the appearance of new lesions and normalization of markers (where applicable), according to the criteria described in the table below. The overall response assessment is shown in the last column, and depends on the assessments of target, non-target, marker and new lesions in the preceding columns.

Target lesion(s)	Non-target lesion(s)	Tumor marker(s)	New lesion(s)*	Bone marrow	Overall response
CR	CR	Normal	No	CR	CR
CR	NE/SD	Normal	No	Not PD	PR
CR	CR, NE, or SD	Abnormal	No	Not PD	PR
PR	CR, NE, or SD	Any	No	Not PD	PR
SD	CR, NE, or SD	Any	No	Not PD	SD
PD	Any	Any	Any	Any	PD
Any	PD	Any	Any	Any	PD
Any	Any	Any	Yes	Any	PD
Any	Any	Any	Any	PD	PD

***New lesions by functional imaging as defined above for PET, MIBG, or bone scan**
NE = not-evaluable

APPENDIX D
DISEASE EVALUATION CRITERIA AND GUIDELINES – ADULT BRAIN TUMORS

I. Recommended Response Criteria for Adult Patients with Brain Tumors

Response Assessment in Neuro-Oncology (RANO) criteria is the recommended criteria to be used to assess radiographic changes in adult patients with brain tumors (Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol. Apr 10 2010;28(11):1963-1972). Data will be used to calculate PFS. In the case of no measurable disease pre-transplant, absence of new disease (see definition in Section XV.B) at each time-point will be documented to determine lack of disease progression.

II. Definitions

A. Complete response requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions; patients must be off corticosteroids (or on physiologic replacement doses only); and stable or improved clinically. In the absence of a confirming scan 4 weeks later, this response will be considered only stable disease

B. Partial response requires all of the following: ≥ 50% decrease, compared with baseline, in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks; no progression of nonmeasurable disease; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; and patient must be on a corticosteroid dose not greater than the dose at time of baseline scan and is stable or improved clinically. In the absence of a confirming scan 4 weeks later, this response will be considered only stable disease.

C. Stable disease occurs if the patient does not qualify for a complete response, partial response, or progression (see letter D) and requires the following: stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan and clinically stable status. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

D. Progression will be defined by any of the following: ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions (compared with baseline if no decrease), on stable or increasing doses of corticosteroids; a significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy not caused by comorbid events; the appearance of any new lesions; clear progression of nonmeasurable lesions; or definite clinical deterioration not attributable to other causes apart from the tumor or to decrease in corticosteroid dose. Failure to return for evaluation as a result of death or deteriorating condition should also be considered as progression.

Increase in corticosteroid dose alone, in the absence of clinical deterioration related to tumor, will note be used as a determinant of progression. Patients with stable imaging studies whose corticosteroid dose was increased for reasons other than clinical deteroiation related to tumor do not qualify for stable disease or progression. They should be observed closely. If their corticosteroid dose can be reduced back to baseline,

they will be considered as having stable disease; if further clinical deteroiation related to tumor becomes apparent, they will be considered to have progression. The date of progression should be the first time point at which corticosteroid increase was necessary.

Patients with nonmeasurable enhancing disease whose lesions have significantly increased in size (minimal bidirectional diameter of ≥10mm and visible on at least two axial slices that are preferably, at most, 5mm apart with 0-mm skip)and become measurable will also be considered to have experienced progression.

The transition from a nonmeasurable lesion to a measurable lesion resulting in progression can theoretically occur with relatively small increases in tumor size. Ideally, the change should be significant (>5mm incrase in maximal diameter or ≥25% increase in sum of the products of perpendicular diameters of enhancing lesions). In general, if there is doubt about whether the lesion has progressed, continued treatment and close follow-up evaluation will help clarify whether there is true progression.

If there is uncertainity regarding whether there is progression, the patient may continue on treatment and remain under close observation. If subsequent evaluations suggest that the patient is experiencing progression, then the date of progression should be the time point at which the issue was first raised.

Summary of the Proposed RANO Response Criteria

Criterion	Complete Response	Partial Response	Stable Disease	Progressive Disease
T1 gadolinium enhancing disease	None	≥50% or Decreasing	<50% Decreasing but <25% Increasing	≥25% Increasing*
T2/FLAIR	Stable or decreasing	Stable or Decreasing	Stable or Decreasing	Increasing*
New Lesion	None	None	None	Present*
Corticosteroids	None	Stable or Decreasing	Stable or Decreasing	NAţ
Clinical Status	Stable or Increasing	Stable or Increasing	Stable or Increasing	Decreasing*
Requirement for Response	All	All	All	Any*

*Progression occurs when this criterion is present.
ţ Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

APPENDIX E
RESEARCH TESTING AND SPECIMENS

Window assessments: Day +7 (Donor: NK product itself; [a]Patient: Morning on the day patient receives NK cell infusion), Day +8[b] (Morning after patient receives NK cell infusion), Day +21 (± 3 days), Day +28 (± 3 days), Day +100 (± 2 weeks), Day +180 (± 2 weeks); 1 year (± 4 weeks)

Research Tests	Donor		Recipient								
	Pre-HCT	Day +7	Pre-HCT	Post-HCT	Day +7[a]	Day +8[b]	Day +21	Day +28	Day +100	Day +180	1 Year
KIR GENOTYPING	X		X								
IMMUNOPHENOTYPING	X		X		X	X	X	X	X	X	X
CYTOKINE PROFILING	X		X		X	X	X	X	X	X	X
NK CYTOTOXICITY ASSAY	X						X	X	X	X	X
NK PRODUCT IMMUNOPHENOTYPING AND CYTOTOXICITY ASSAY		X									
HRQoL SURVEY			X					X	X	X	X
TUMOR SAMPLE			X	X							
GVHD SAMPLE				X							

TEST	SAMPLE REQUIREMENTS	LABORATORY
KIR genotyping	10 mL blood in EDTA tube	Blood Center of Wisconsin
Immunophenotyping (includes KIR phenotyping)	10 mL blood in Sodium Heparin tube (for both tests) (serum will be spun out for cytokine studies)	Malarkannan/Margolis Lab
Cytokine profiling		
NK Cytotoxicity Assay	10 mL in Sodium Heparin tube	Malarkannan/Margolis Lab
NK Product Immunophenotyping and Cytotoxicity Assay	10 x 10^6 NK cells from final NK product	Malarkannan/Margolis Lab
[1] Health-Related Quality of Life Survey (SEE TABLE NEXT PAGE)	Child Health Rating Inventories (CHRIs) for pediatric patients) -or- the Functional Assessment of Chronic Illness Therapy (FACIT) and Patient Reported Outcomes Measurement Information System (PROMIS) for adult patients	N/A; Survey to be provided by CRC to patient or family
Tumor Sample	Paraffin-embedded sample at time of diagnosis (if available) in Biobank Repository for immunostaining and/or any post-transplant tumor staging biopsy samples	Malarkannan/Margolis Lab
GVHD Sample	Paraffin-embedded sample of any tissue (if available) at time of suspicion of GVHD	Malarkannan/Margolis Lab

APPENDIX E, CON'T:
[1] Health-Related Quality of Life (HRQL) Assessments

Age group-specific (school-aged, adolescent, and adult) assessments, and when using CHRIs, rater-specific [parent vs. child] HRQL assessments, will be collected prior to and at day +28, +100, +180, and 12 months post-transplant to document each patient's HRQL before and following HCT. Assessment windows have been created at each time period (see preceding chart for windows allowed). Dr. Parsons and Dr. Bingen are co-investigators who will additionally aid in data interpretation.

CHRIs

Eligible child participants with adequate cognitive functioning (FS IQ≥ 70 on baseline neurocognitive testing will complete the Child Health Ratings Inventories (CHRIs)-General Health Module immediately following neurocognitive testing under the supervision of the psychologist. The CHRIs-General contains 20 items assessing the child's functioning in the areas of physical, role, and emotional functioning. A 5-point, Likert-style response scale is presented for each item. The reference period is one week. Domain scores are scaled from 0-100, using established scoring algorithms, in which higher scores connote better functioning.

As noted, neurocognitive testing and HRQL assessment must be completed within one month of beginning therapy and in all cases, before the start of the preparative regimen. Children 5-12 years will complete the school-aged version of the CHRIs-General; adolescents 13-18 years will complete the adolescent version. An abbreviated version of the parental measure for children <5 yrs is also available. Throughout the study, the child participant will complete the same version of the CHRIs, even if they "age out" of an age category. If the child turns 18 years during the course of the study, he/she will be re-consented.

Parent participants will complete the corresponding rater-specific version of the child measure ("Parent of School-aged Child" or "Parent of Adolescent). The parent will complete the baseline assessment while the child is completing the neurocognitive battery. Of note, one parent participant must be designated per family at study entry. No substitutions throughout the study will be permitted. The participating parent must have residential contact with the child and have legal authority to provide consent on behalf of the minor child.

At each of the follow-up time periods, both raters will complete the CHRIs-General, following in all cases by the CHRIs-HSCT, a 10-item, HSCT-specific module, which forms three domains: hassles; distress; and body image. The 5-point Likert scale also is used with the CHRIs-HSCT with the same one-week reference period. The two instruments take approximately 10-15 minutes to complete.

All assessments will be preassembled and distributed to the study sites by the central HRQL site (Tufts). All assessments will contain a de-identified dyad number (study ID plus the following digit, 1 for parent report, 2 for child report [i.e. 526-001-1, 526-001-2) and date of completion. Once completed, the site will make a photocopy of the entire packet and retain the copy for their study files. The original will be mailed via traceable carrier to the Tufts site, where the data will be reviewed for data quality. Ambiguities will be directed, where possible, to the sites for clarification. The data will then be double data entered into a web-based, password- protected SQL server. Data will be scored by dyad and provided in a de-identified data file to the PI, David Margolis, MD, for further analysis.

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(Notify by email sparsons@tuftsmedicalcenter.org when packet is being sent)

FACIT

The Functional Assessment of Cancer Therapy-Bone Marrow Transplantation (FACT-BMT) is a well-validated, self-report instrument measuring health-related quality of life in adults. The FACT-BMT consists of four general HRQL domains derived from the FACT-General (FACT-G): (1) Physical well-being (PWB, 7-items), (2) Emotional well-being (EWB, 6-items), (3) Social/family well-being (SWB, 7-items) and (4) Functional well-being (FWB, 7-items). It also includes a fifth domain of 23 supplemental items that are specific to BMT (BMT Subscale). Additional scores that may be derived in addition to the 5 domain scores include: (1) FACT-BMT Trial Outcome Index (PWB+FWB+BMT); (2) FACT-G Total Score (PWB+SWB+EWB+FWB); and (3) FACT-BMT Total Score (PWB+SWB+EWB+FWB+BMT). The questionnaire is written at the 4th grade reading level and is able to be completed in 5-10 minutes. It is reverse-scored such that the higher the score, the better the HRQL. The FACT-G/FACT-BMT has well-established psychometrics including reliability, validity, and sensitivity to change. After registration, all surveys, scoring, and interpretation materials are publically available on-line from http://www.facit.org.

PROMIS

The PROMIS project is a NIH Roadmap Initiative to create standardized patient-reported outcomes tools used in health and QOL clinical research and practice. Using a comprehensive quantitative and qualitative methodological approach to instrument development, PROMIS item banks and measures of salient symptoms and domains of health, or "Short Forms" (developed from the item banks), have been created and validated. The PROMIS Global Health instrument is a short-form scale consisting of 10 items which are scored into physical and mental health summary scores. It uses a 5-point Likert scale with a response period of "the past 7 days." The higher the score, the better the quality of life. It takes about 5 minutes to complete. After registration, all surveys, scoring, and interpretation materials are publically available from http://www.nihpromis.org.

SURVEY	Children and Young Adults* treated at the Children's Hospital		Young Adults* and Adults treated at the Adult Hospital	
	Baseline	Post-HCT timepoints	Baseline	Post-HCT timepoints
CHRIs Assessments				
Demographics	X		X	
General Health Module**	X	X		
HSCT**		X		
Parent Report***	X	X		
FACIT Assessment				
FACT-G			X	
FACT-BMT (includes FACT-G)				X
PROMIS Assessment				
PROMIS-Global Health			X	X

* Young Adults are a challenging age group to test due to situational issues such as emancipation and emotional maturity. Thus, those young adults primarily being treated at the Children's Hospital will be tested using CHRIs-based surveys while young adults primarily treated at the Adult Hospital will be tested using FACIT/PROMIS-based surveys. *For example, an 18 year old patient being primarily treated at the adult hospital will use FACIT/PROMIS-based surveys.* CHRIs demographics form will be used for all patients regardless of age.

** Children 8-12 years: Fill out "School-aged" version of CHRIs forms
** Adolescents 13-18 years: Fill out "Adolescent" version of CHRIs forms
*** All parents (regardless of child's age) will fill out a Parent Report which includes parent proxy questions.

APPENDIX F
GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE[1]

Severity of Individual Organ Involvement (Stage)

Body Area	Percent
Each Arm	9%
Each Leg	18%
Chest & Abdomen	18%
Back	18%
Head	9%
Pubis	1%

Skin

0	No rash or rash not attributable to GVHD
+1	Maculopapular eruption involving less than 25% BSA
+2	a maculopapular eruption involving 25-50% of BSA
+3	generalized erythroderma (i.e, >50% of BSA)
+4	generalized erythroderma with bullous formation and often with desquamation

Liver

0	Bilirubin (<2.0mg/dL)
+1	Bilirubin (2.0-3.0 mg/dL)
+2	Bilirubin (3.1-6.0 mg/dLl)
+3	Bilirubin (6.1-15 mg/dL)
+4	Bilirubin > 15 mg/dL

Gut

Nausea and vomiting and/or anorexia caused by GVHD is assigned as +1 in severity. The severity of gut involvement is assigned to the most severe involvement noted. Patients with visible bloody diarrhea are at least stage +2 gut and grade +3 overall Diarrhea as seen in the absence of infectious/medical causes.

Stage	Age <16 yrs old	Age >16 yrs old
0	No diarrhea	No diarrhea
0	Diarrhea <280 ml/m2/day	Diarrhea <500 mL/day
1	Diarrhea 280-555 ml/m2/day	Diarrhea >500 but <1000ml/day
1	Persistent nausea and vomiting (no diarrhea)	Persistent nausea and vomiting (no diarrhea)
2	Diarrhea 556-883 ml/m2/day	Diarrhea >1000 but <1500mL/day
3	Diarrhea >883 ml/m2/day	Diarrhea >1500mL/day
4	Severe Abdominal pain, with or without ileus	Severe Abdominal pain, with or without ileus

Severity of Overall GVHD (Grade)

Grade I
+1 to +2 skin rash
No gut or liver involvement

Grade II
+1 to +3 skin rash and/or
+1 gastrointestinal involvement and/or +1 liver involvement

Grade III
+1 to +3 skin rash and/or
+2 to +4 gastrointestinal involvement and/or
+2 to +3 liver

Grade IV
+4 skin rash and/or
+4 liver and/or extreme decrease in clinical performance or death

[1] Reference: Post-TED (Form2450) v2 (8/1/2012)

APPENDIX G
EVALUATION OF CHRONIC GRAFT-VERSUS-HOST DISEASE[1]

Grading of Chronic GVHD

Limited: Localized skin involvement resembling localized scleroderma with or without liver involvement; no other organ involvement.
Extensive: Generalized skin and/or multiple organ involvement.

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. Karnofsky or Lansky Clinical Performance scores ≤ 60%, >15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ systems as listed below:

Skin	Erythema, dryness, pruritus, pigmentary changes (i.e. hyperpigmentation, vitiligo), mottling, papulosquamous plaques, nodules, exfoliation, macular-papular or urticarial rash, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)
Nails	Ridging, onychodystrophy, onycholysis
Hair	Premature graying, (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair
Mouth	Dryness, burning, gingivitis, mucositis, striae, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tooth decay, tightness around the mouth
Eyes	Dryness, burning, blurring, gritty eyes, photophobia, pain
Vagina/vulva	Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not included
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase ≥ 3x upper limit of normal, AST or ALT ≥4x upper limit of normal or total serum bilirubin ≥ 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures
Serositis	Chest pain or cardiopulmonary comprise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

Laboratory Testing and Diagnostic Indicators of Chronic GVHD

Eye	Schirmer's test with a mean value ≤ 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as FEV_1 < 80% of predicted with either an FEF_{25-75} <65% of predicted or RV >120% of predicted, or a decrease of FEV_1/FVC by > 12% within a period of less than 1 year. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans o the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/μl), eosinophilia, hypogammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

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[1] From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

APPENDIX H
Adapted from COMMON TOXICITY CRITERIA (CTC) - Version 4.0

	Grade		
Adverse Event	**3**	**4**	**5**
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Disseminated intravascular coagulation	Laboratory findings and bleeding	Life-threatening consequences; urgent intervention indicated	Death
Febrile neutropenia	ANC <1000/mm3 with a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4 F) for more than one hour	Life-threatening consequences; urgent intervention indicated	Death
Hemolysis	Transfusion or medical intervention indicated (e.g., steroids)	Life-threatening consequences; urgent intervention indicated	Death
Hemolytic uremic syndrome	Laboratory findings with clinical consequences (e.g., renal insufficiency, petechiae)	Life-threatening consequences, (e.g., CNS hemorrhage or thrombosis/embolism or renal failure)	Death

	Grade		
Adverse Event	**3**	**4**	**5**
CARDIAC DISORDERS			
Atrial fibrillation	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
Atrial flutter	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
Atrioventricular block complete	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening consequences; urgent intervention indicated	Death
Constrictive Pericarditis	Symptomatic heart failure or other cardiac symptoms, responsive to intervention	Refractory heart failure or other poorly controlled cardiac symptoms	Death
Heart failure	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated	Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)	Death
Constrictive Pericarditis	Symptomatic heart failure or other cardiac symptoms, responsive to intervention	Refractory heart failure or other poorly controlled cardiac symptoms	Death
Heart failure	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated	Life-threatening consequences; urgent intervention indicated (e.g. cont IV therapy or mechanical hemodynamic support)	Death

Left ventricular systolic dysfunction	Symptomatic due to drop in ejection fraction responsive to intervention	Refractory or poorly controlled heart failure due to drop in ejection fraction; intervention such as ventricular assist device, intravenous vasopressor support, or heart transplant indicated	Death
Myocardial infarction	Severe symptoms; cardiac enzymes abnormal; hemodynamically stable; ECG changes consistent with infarction	Life-threatening consequences; hemodynamically unstable	Death
Myocarditis	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated	Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)	Death
Pericardial effusion	Effusion with physiologic consequences	Life-threatening consequences; urgent intervention indicated	Death
Pericardial tamponade	-	Life-threatening consequences; urgent intervention indicated	Death
Ventricular arrhythmia	Medical intervention indicated	Life-threatening consequences; hemodynamic compromise; urgent intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
GASTROINTESTINAL DISORDERS			
Ascites	Severe symptoms; invasive intervention indicated	Life-threatening consequences; urgent operative intervention indicated	Death
Diarrhea	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Duodenal ulcer	Severely altered GI function; TPN indicated; elective operative or endoscopic intervention indicated; limiting self care ADL; disabling	Life-threatening consequences; urgent operative intervention indicated	Death
Gastric ulcer	Severely altered GI function; TPN indicated; elective operative or endoscopic intervention indicated; limiting self care ADL; disabling	Life-threatening consequences; urgent operative intervention indicated	Death
Gastritis	Severely altered eating or gastric function; TPN or hospitalization indicated	Life-threatening consequences; urgent operative intervention indicated	Death
Lower gastrointestinal hemorrhage	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Mucositis oral	Severe pain; interfering with oral intake	Life-threatening consequences; urgent intervention indicated	Death
Oral hemorrhage	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Pancreatitis	Severe pain; vomiting; medical intervention indicated (e.g.,	Life-threatening consequences; urgent intervention indicated	Death

	analgesia, nutritional support)		
Typhlitis	Symptomatic (e.g., abdominal pain, fever, change in bowel habits with ileus); peritoneal signs	Life-threatening consequences; urgent operative intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
GENERAL DISORDERS AND ADMINISTARTION SITE CONDITIONS			
Multi-organ failure	Shock with azotemia and acid-base disturbances; significant coagulation abnormalities	Life-threatening consequences (e.g., vasopressor dependent and oliguric or anuric or ischemic colitis or lactic acidosis)	Death

	Grade		
Adverse Event	**3**	**4**	**5**
HEPATOBILIARY DISORDERS			
Cholecystitis	Severe symptoms; radiologic, endoscopic or elective operative intervention indicated	Life-threatening consequences; urgent operative intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
IMMUNE SYSTEM DISORDERS			
Allergic reaction	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated	Death
Immune system disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
	Grade		
Adverse Event	**3**	**4**	**5**
INFECTIONS AND INFESTATIONS			

Enterocolitis infectious	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic, endoscopic, or operative intervention indicated; profuse watery diarrhea with signs of hypovolemia; bloody diarrhea; fever; severe abdominal pain; hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Infections and infestations - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
INVESTIGATIONS			
Alanine aminotransferase increased	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Aspartate aminotransferase increased	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Blood bilirubin increased	>3.0 - 10.0 x ULN	>10.0 x ULN	-
Carbon monoxide diffusing capacity decreased	Asymptomatic decrease of >8 units drop; >5 units drop along with the presence of pulmonary symptoms (e.g. , >Grade 2 hypoxia or >Grade 2 or higher dyspnea)	-	-
Cardiac troponin I increased	Levels consistent with myocardial infarction as defined by the manufacturer	-	-
Cardiac troponin T increased	Levels consistent with myocardial infarction as defined by the manufacturer	-	-
Creatinine increased	>3.0 baseline; >3.0 - 6.0 x ULN	>6.0 x ULN	-
Weight gain	>=20% from baseline	-	-

	Grade		
Adverse Event	**3**	**4**	**5**
METABOLISM AND NUTRITIONAL DISORDERS			
Hypercalcemia	Corrected serum calcium of >12.5 - 13.5 mg/dL;>3.1 - 3.4 mmol/L; Ionized calcium >1.6 - 1.8 mmol/L; hospitalization indicated	Corrected serum calcium of >13.5 mg/dL; >3.4 mmol/L; Ionized calcium >1.8 mmol/L; life-threatening consequences	Death
Hypertriglyceridemia	>500 mg/dL - 1000 mg/dL; >5.7 mmol/L - 11.4 mmol/L	>1000 mg/dL; >11.4 mmol/L; life-threatening consequences	Death

Hyperuricemia	>ULN - 10 mg/dL (0.59 mmol/L) with physiologic consequences	>10 mg/dL; >0.59 mmol/L; life-threatening consequences	Death
Tumor lysis syndrome	Present	Life-threatening consequences; urgent intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
NEOPLASMS BENIGN, MALIGNANT, AND UNSPECIFIED (INC CYSTS AND POLYPS)			
Treatment related secondary malignancy	Non life-threatening secondary malignancy	Acute life-threatening secondary malignancy; blast crisis in leukemia	Death

	Grade		
Adverse Event	**3**	**4**	**5**
NERVOUS SYSTEM DISORDERS			
Dysarthria	Severe impairment of articulation or slurred speech	-	-
Intracranial hemorrhage	Ventriculostomy, ICP monitoring, intraventricular thrombolysis, or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Ischemia cerebrovascular	-	-	-
Leukoencephalopathy	Severe symptoms; extensive T2/FLAIR hyperintensities, involving periventricular white matter involving 2/3 or more of susceptible areas of cerebrum +/- moderate to severe increase in SAS and/or moderate to severe ventriculomegaly	Life-threatening consequences; extensive T2/FLAIR hyperintensities, involving periventricular white matter involving most of susceptible areas of cerebrum +/- moderate to severe increase in SAS and/or moderate to severe ventriculomegaly	Death
Seizure	Multiple seizures despite medical intervention	Life-threatening; prolonged repetitive seizures	Death
Syncope	Fainting; orthostatic collapse	-	-
Nervous system disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
RENAL AND URINARY DISORDERS			

Chronic kidney disease	eGFR or CrCl 29 - 15 ml/min/1.73 m2	eGFR or CrCl <15 ml/min/1.73 m2; dialysis or renal transplant indicated	Death
Renal and urinary disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

Grade			
Adverse Event	**3**	**4**	**5**
RESPIRATORY, THORACIC, AND MEDIASTINAL DISORDERS			
Adult respiratory distress syndrome	Present with radiologic findings; intubation not indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Apnea	Present; medical intervention indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Bronchopulmonary hemorrhage	Transfusion, radiologic, endoscopic, or operative intervention indicated (e.g., hemostasis of bleeding site)	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Hypoxia	Decreased oxygen saturation at rest (e.g., pulse oximeter <88% or PaO2 <=55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Pleural effusion	Symptomatic with respiratory distress and hypoxia; surgical intervention including chest tube or pleurodesis indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Pneumonitis	Severe symptoms; limiting self care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Pulmonary edema	Severe dyspnea or dyspnea at rest; oxygen indicated; limiting self care ADL	Life-threatening respiratory compromise; urgent intervention or intubation with ventilatory support indicated	Death
Respiratory failure	-	Life-threatening consequences; urgent intervention, intubation, or ventilatory support indicated	Death
Grade			
Adverse Event	**3**	**4**	**5**
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Erythema multiforme	Target lesions covering >30% BSA and associated with oral or genital erosions	Target lesions covering >30% BSA; associated with fluid or electrolyte abnormalities; ICU care or burn unit indicated	Death
Grade			

Adverse Event	3	4	5
VASCULAR DISORDERS			
Capillary leak syndrome	Severe symptoms; intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Hypotension	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated	Death
Thromboembolic event	Thrombosis (e.g., uncomplicated pulmonary embolism [venous], non-embolic cardiac mural [arterial] thrombus), medical intervention indicated	Life-threatening (e.g., pulmonary embolism, cerebrovascular event, arterial insufficiency); hemodynamic or neurologic instability; urgent intervention indicated	Death
Vasculitis	Severe symptoms, medical intervention indicated (e.g., steroids)	Life-threatening; evidence of peripheral or visceral ischemia; urgent intervention indicated	Death

APPENDIX I
STUDY COORDINATOR'S MANUAL

I. Introduction

The study coordinator procedure manual was created to assure consistency of data reporting across the centers and to assure compliance with regulations. General expectations of collaborators are that they will comply with appropriate regulatory requirements, specified protocol requirements, and provide outcome data.

The manual translates working procedures for study coordination. Its goal is to describe the procedures with sufficient clarity to ensure that all study centers will use the same procedures and follow-up schedules for participant data management and reporting. Changes to the manual and relevant forms will be made as soon as practical and will become effective on receipt of the revised procedures at the study centers, unless otherwise noticed.

II. Institutional Review Board Review of Protocols and Modifications

All research protocols proposed for use that involves human subjects must be reviewed and approved by the Institutional Review Board (IRB) prior to implementation. New protocols will undergo review at the CHW IRB and then will be distributed to sites that wish to participate for their local IRB's review. For Centers that have a Federal Wide Assurance (FWA), formal collaboration includes submission of a form 310 and a copy of the IRB approved protocol and IRB approved consent forms to the coordinating site (CHW). For sites without a FWA, an FWA form needs to be filed. Once the paperwork is submitted to the Office for Human Research Protection, the approval process can take up to a couple of months, and must be completed before collaboration on a protocol can begin.

In addition, all amendments and/or revisions to on-going, approved activities must be submitted for review and approved prior to implementation at an institution. No revisions may be implemented at outside institutions without the prior approval of the CHW Principal Investigator. The CHWIRB and the local site's IRB must review all protocol activities at least once annually. This must be done within 365 days of the last review regardless of the policies of the institution. A copy of annual renewal approvals must be received for collaboration to continue for the next year.

III. Registrations

Collaborating Institutions: The principal investigator of the collaborating institution who will register the patient with CHW will identify eligible patients. Registration will include completion of the eligibility checklist/demographic form. This form will be faxed (414-955-0116) prior to treatment initiation. An email should also be sent tocrconc@mcw.edu when faxing the registration information. Patients should be registered prior to treatment initiation for valid registration.

IV. Reporting Adverse Events

The guidelines of **Section XVI** are the minimum serious adverse event (SAE) reporting guidelines. Please see **Section XVI.B** for Adverse Event definitions. Collaborating institutions will follow all reporting guidelines as indicated in the protocol.

Expedited Reporting Requirements

All unexpected and serious adverse events which may be related to study treatment or intervention must be reported to the CHW Clinical Trials Office within 48 hrs and to the CHW IRB as soon as possible but within

at least 5 calendar days of the investigator learning of the event. All deaths, regardless of the cause, need to be reported to the coordinating center PI within 48 hours and are then reported to the IRB.

Unexpected Adverse Event – An adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product). If applicable product information is not available, such as for studies that do not involve pharmaceutical products or devices, an unexpected adverse event is an adverse event that was not described in the study protocol or informed consent.

Serious Adverse Event (SAE) – Any adverse event occurring that results in any of the following outcomes:

1) Death – start of any protocol intervention to day 180, regardless of cause,
2) a life-threatening adverse event (see above)
3) a persistent or significant disability/incapacity
4) a congenital anomaly that requires intervention to prevent permanent impairment or damage.

To ensure no confusion or misunderstanding exist of the differences between the terms "serious" and "severe," which are not synonymous the following note of clarification is provided:

- ***The term "severe" is often used to describe the intensity (severity) or a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory obligations.***

Attribution – The designation for the determination of whether an adverse event is related to a medical product, treatment or procedure will be as follows:

1) **Related** – includes adverse events that are definitely, probably, or possibly related to the medical treatment or procedure.

2) **Not Related** – includes adverse events are doubtfully related or clearly not related to the medical treatment or procedure.

Each institution's Serious Adverse Event (SAE) Report Form should be completed for all adverse events that meet the expedited reporting requirements. All available information should be submitted but it is acceptable to fax an incomplete report form at the initial report. A completed report should be faxed as soon as possible but must be received within 5 calendar days.

It is the responsibility of the CHW Principal Investigator to notify NIH and the responsibility of the IND holder to notify the FDA or other agencies of serious adverse events as required in the protocol.

Serious adverse events that do not meet the requirement for expedited reporting (not related to study treatment or expected) will be reported to the IRB as part of the annual renewal of the protocol.
CHW is acting as the Coordinating Center for this multi-institutional study, and it is the responsibility of the CHW Principal Investigator (or designee) to accurately report all serious adverse events that meet the expedited reporting requirements that are received from the participating sites.

Procedure for Reporting Serious and Unexpected Adverse Events from Participating Sites

Regulations defining the responsibilities for reporting serious and unexpected adverse reactions are defined above. Serious and unexpected adverse events must be reported to the CHW Investigator and the IND holder within 48 hours of learning of the event. This includes patient deaths, regardless of cause, occurring at the start of any protocol intervention and day 180 post-transplant procedure. The immediate telephone report must be followed by a faxed report to the CHW Trial Coordinator at (414-955-0116). Participating sites may complete and fax their local IRB report forms. The CHW Trial Coordinator will then complete the CHW detailed IRB report form within 3 additional working days. All reports must include the date and time of onset, date the PI became aware of the event, severity and duration of the event, the relationship to the study, the treatment given and eventual outcome. Dr. Margolis and the coordinating center will ensure that the SAE is forwarded to the CHW IRB, the MCW Cancer Center DSMB and the FDA. Follow-up information to a SAE report must be submitted as soon as the relevant information is available.

This information should also be entered in the EDC, but de-identified paper report forms must also be sent to Dr. Margolis as described in the protocol.

Obligation of Investigators

All grade 3 or 4 adverse events, or highly unusual grade 2 adverse events, using the modified (for HSCT) NCI Common Toxicity Criteria v.4.0 which occur between the start of any protocol intervention and day 100 during the study will be recorded on the Case Report Form. These adverse events which are observed by the Investigator or reported by the patient whether or not attributed to the study, will be recorded on the Case Report Form. Attributes will include a description, date of onset, maximum severity, and assessment of relationship to the study agent or other suspect agent(s).

Adverse events will be graded accordingly: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life threatening or debilitating, and 5 = fatal. All Grade 4 (life-threatening) or Grade 5 (fatal) events on the adapted HSCT NCI scale meet expedited reporting requirements.

Association or relatedness to the study agent will be graded as follows: 1 = unrelated, 2 = unlikely, 3 = possibly, 4 = probably, and 5 = definitely related.

V. Case Report Forms

Electronic or paper case report forms must be completed for all patients registered onto the protocols and submitted to the CHW Clinical Trials Office. The first case report form (day 28) is due on day 50. EDC forms will be completed for day 28, day 56, day 84, and day 100 post-transplant, as well as day 180, 1 year, 1.5 years, 2 years, and yearly thereafter. Case report forms are expected to be submitted no later than 30 days following the scheduled follow up date.

VI. Protocol Monitoring

As the coordinating center, CHW will monitor accrual at the outside institutions. The guidelines below are intended to guide the reviewers in their assessment of items that significantly alter the clinical effectiveness of the treatment or the evaluation of its toxicity.

A. Registration/Randomization
   1. Patient was registered prior to treatment and approval by CHW PI occurs prior to randomization.
   2. Information given at registration represents actual data in medical records (stage, diagnosis, cell type, etc.)

B. Informed Consent/IRB Approval Dates
   1. The consent was signed prior to registration.
   2. The consent is in language was approved by the institution's IRB. IRB approval and re-approval are documented including appropriate use of full-board review and proper review of appropriate amendments or revisions
   3. Consent was dated and has written witness signature. IRB approval was obtained prior to the patient signing the consent form and start of treatment.

C. Patient Eligibility
   1. Eligibility criteria and exclusion criteria were met.
   2. Treatment/Intervention Administration
   3. Doses were modified according to protocol
   4. Accurate documentation of drug administration

D. Study Tests/Evaluation
   1. Protocol specified laboratory tests or diagnostic studies are available
   2. Appropriate record of protocol intervention is documented.

E. Study Events/Adverse Drug Experience
   1. Serious Adverse Events reported according to protocol specifications

F. Follow-Up
   1. Disease status assessed according to the required protocol guidelines documenting response to treatment.
   2. Accurate determination of cancer progression

APPENDIX J
COORDINATING CENTER FUNCTIONS

I. Study Management, Data analysis, and Data and Safety Monitoring

a. Study Management:
   i. Each local PI is responsible for selection, training and oversight of local study coordinators.
   ii. Dr. Margolis will hold a start-up teleconference(s) with the collaborating sites prior to patient enrollment.
   iii. Once a collaborating institution consents a patient, the center will complete the Demographics form, Registration form, HCT-Comorbidity Index on the paper case report forms. The coordinating site will assign a sequential study ID. Collaborating Centers will be required to upload the signed informed consent and de-identified patient source documentation at time of registration. The Coordinating Center will review the Registration within 24-72 hours of submission and will notify the collaborating site of registration approval prior to start of patient conditioning.
   iv. Subsequent data including baseline disease history form will be entered via the OnCore™ database management system for review by the collaborating center in the same manner. The coordinating center will follow-up with the collaborating institution if there are missing or delinquent data forms, and request submission within a timely manner.
   v. One copy of the research data is retained by the collaborating center. Another data set (identified only by study IDs) is transmitted to the Coordinating Center via OnCore™ to create the master data file. Any paper data are kept in locked areas and password protected databases accessible only to study staff
   vi. The quality of data is monitored in an ongoing fashion with the study team and corrective action plans instituted as necessary.

b. Data Analysis:
   i. Study staff review data for completeness as it is submitted by the sites
   ii. The study statistician is responsible for data cleaning and the conduct of analyses as outlined in the protocol and grant.

c. Data Safety and Monitoring:
   i. The trial coordinators at collaborating centers or the local PIs will fax an official report of an SAE to the Coordinating Center (CHW) within 48 hours if the event occurred during the initial 28 days post NK infusion, otherwise within five days.
   ii. The SAE report is reviewed by the Overall PI. If the SAE meets the CHW criteria for reporting then an official signed report is submitted to the IRB.
   iii. An independent DSMB will meet at six-month intervals and all outcome data is reviewed including all adverse events and SAEs reported to the Coordinating Center along with those officially reported to the IRB.
   iv. A report from the DSMB is submitted to the IRB as well as the trial coordinators/ local PIs participating in the protocol.

II. Protocol and informed consent document management

a. A master protocol is maintained by the Coordinating Center and distributed to the sites for customization and local IRB review.
b. All protocol and consent modifications initiated by the Coordinating Center are sent to the Collaborating Sites following approval by the Coordinating Center IRB, for review and approval by the local IRB

c. Changes required by local IRBs are reviewed by the Coordinating Center and approved prior to implementation at local sites

III. Assurance of local IRB OHRP-approved assurance

a. Each site provides their OHRP assurance number and evidence of IRB certification
b. Study staff monitor maintenance of institutional assurance and IRB certification

IV. Assurance of local IRB approvals

a. The Coordinating Center maintains copies of the most current collaborating site Consent Forms and IRB approval documentation
b. No site may enroll subjects until the Coordinating Center has received confirmation of local IRB approval
c. Each site is responsible for preparation and submission of their continuing reviews. Any changes to the protocol or consent form will be communicated to the Coordinating Center
d. Sites are required to have active IRB approvals to participate in any study related activities

V. Any substantive modification by the Collaborating Institution related to risks or alternative procedures is appropriately justified

a. The Coordinating Center reviews any modifications to consent forms to ensure that site consents do not delete or change the basic or additional elements or alternatives required in the sample consent form

VI. Informed consent is obtained from each subject in compliance with HHS regulations

a. Subjects must provide written informed consent prior to study participation
b. The Coordinating Center verifies eligibility and signed consent prior to assigning a study ID number

APPENDIX K
STIR Protocol - PATIENT DEMOGRAPHICS AND REGISTRATION FORM

Please complete and fax this completed form to (414)-955-0116 for patient registration. The form can also be emailed to otarnowske@mcw.edu & tpack@chw.org

For questions regarding eligibility please contact David Margolis, M.D., 414-955-4195.

UPN: _____(will be assigned by coordinating center)

Patient Name: _____ _____ _____
(Last) *(First)* *(MI)*

Date of Birth: _____ / _____ / _____ **Age**:_____
(Mo) (Day) (Year)

Gender *(choose one)*:
☐ Male ☐ Female ☐ Unknown

Patient Diagnosis: _____

Planned Day 0: _____/_____/_____
(Mo) (Day) (Year)

Ethnicity *(choose one)*: *Instruct the patient to select one of the following.*

- ☐ **Hispanic** *(A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. Term "Spanish Origin" can also be used in addition to "Hispanic" or "Latino".)*
- ☐ **Not Hispanic or Latino**
- ☐ **Declined to Report**

Race *(check all that apply)*: *Instruct the patient to select one or more of the following.*

- ☐ **American Indian/Alaska Native** *(A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment).*
- ☐ **Asian** *(A person having origins in any of the original peoples of the Far East, Southeast, Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand and Vietnam).*
- ☐ **Black/African American (***A person having origins in any of the black racial groups of Africa).*
- ☐ **Native Hawaiian/Pacific Islander** *(A person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands).*
- ☐ **White** *(A person having origins in any of the original peoples of Europe, the Middle East or North Africa).*
- ☐ **Research subject does not know race**
- ☐ **Declined to report**

Creatinine Clearance:_____ **Date:** _____

Fludarabine Dose: _____

Signature of Principal Investigator: _____ **Date:** _____

Protocol Eligibility

Inclusion Criteria

#1 and #2 of the following Inclusion Criteria must be marked "Yes" for the patient to enroll on study.

Inclusion Note: There is no age restriction.

1. Disease Status
   Yes ☐ No ☐ Patient must have stable disease for at least 3 weeks prior to enrollment.

2. Patient has a disease that is eligible Yes ☐ No ☐ (Must answer YES to one of the following):
   a. Yes ☐ No ☐ High Risk Neuroblastoma (NB): Must have progressed on or recurred after standard frontline therapy including autologous HCT, or be ineligible for autologous HCT.
   b. Yes ☐ No ☐ Ewing Sarcoma Family of Tumors (EWS) [includes both bone and soft tissue Ewing and Peripheral Primitive Neuroectodermal Tumors (PNET)]. Must have progressed on or recurred after standard frontline therapy which includes doxorubicin and ifosfamide.
   c. Yes ☐ No ☐ High-Risk Rhabdomyosarcoma (RMS) or Intermediate Risk Alveolar RMS recurring as more than loco-regional tumor: Must have progressed on or recurred after standard frontline therapy which includes chemotherapy with vincristine, actinomysin, and cyclophosphamide AND either surgery or radiotherapy.

3. Yes ☐ No☐ Osteosarcoma: Must have progressed or recurred after standard frontline therapy. If first relapse, must have recurred with a) ≥ 4 lung nodules; b) bilateral lung involvement; or c) relapse outside the lungs.

4. Yes ☐ No ☐ CNS tumors: High risk malignant brain tumors that are recurrent or refractory to standard frontline therapy are eligible. Diagnoses include: Medulloblastoma, primitive neuro-ectodermal tumor (PNET), ependymoma, high grade (grade 3 or 4) glioma/astrocytoma, germ-cell tumor, or atypical teratoid-rhabdoid tumor (ATRT).

Exclusion Criteria

Each of the following questions must be marked "No" Or "NA" for the patient to enroll on study

1. Yes ☐ No ☐ Patient has rapidly-progressing disease prior to HCT, defined as clinical or radiographic evidence of disease progression ≤ 3 weeks prior to protocol registration despite previous achievement of stable or no disease (Note: additional imaging studies are not necessary unless there are clinical concerns).

2. Yes ☐ No ☐ Patient has reached radiation threshold limits and is excluded from receiving 3 Gy TBI.

3. Yes ☐ No ☐ Diffuse intrinsic pontine gliomas (DIPG) diagnosis.

4. Yes ☐ No ☐ Performance status: Karnofsky or Lansky < 60%
   Note: Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score

5. Yes ☐ No ☐ Patient, in the opinion of the investigator, may not be able to comply with the treatment plan or safety monitoring requirements of the study

6. Yes ☐ No ☐ Significant organ dysfunction that would prevent compliance with conditioning, GVHD prophylaxis, or would severely limit the probability of survival, defined as:

   a. Cardiac: Symptomatic coronary artery disease or ejection fraction < 35% or other cardiac failure requiring therapy (or, if unable to obtain ejection fraction, shortening fraction of < 26%). If shortening fraction is < 26% a cardiology consult is required with the PI having final approval of eligibility

   b. Pulmonary: DLCO < 40% TLC < 40%, FEV1 < 40% and/or receiving supplementary continuous oxygen

   c. Liver: Patient with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, bridging fibrosis, and the degree of portal hypertension. The patient will be excluded if he/she is found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3mg/dL, or symptomatic biliary disease

7. Yes ☐ No ☐ Patient has serious active infections

8. Yes ☐ No ☐ Patient is HIV seropositive

9. Yes ☐ No ☐ Patient has poorly controlled hypertension despite multiple antihypertensive medications.

10. Yes ☐ No ☐ NA ☐ Fertile female who is unwilling to use contraceptive techniques during and for the twelve months following treatment, and/or a female who is pregnant or actively breast feeding

11. Yes ☐ No ☐ NA ☐ Fertile male who is unwilling to use contraceptive techniques during and for the twelve months following treatment

12. Yes ☐ No ☐ Patient's life expectancy is severely limited by diseases other than malignancy

13. Yes ☐ No ☐ Patient has received a prior allogeneic HCT

Note: HCT-Comorbidity Score is: _____

☐ **CHW Patients:**

Signature of person completing form: _____ Date: _____

Patient signed IRB approved consent form. Date: _____

CHW IRB file number: _____ Date of IRB approval: _____

Signature of Principal Investigator: _____ Date: _____
(post signing of consent)

☐ **Outside Center Patients:**

Signature of person completing form: _____ Date: _____

Patient signed IRB approved consent form. Date: _____

IRB file number: _____ Date of IRB approval: _____

Signature of **Local** Principal Investigator _____ Date: _____

Signature of **CHW** Principal Investigator _____ Date: _____

APPENDIX L
DSMB ROLE AND RESPONSIBILITIES

I. Overview

The DSMB will review the progress of the trial following subject enrollment to ensure that patient safety is not being compromised. The DSMB will meet twice a year (approximately every 6 months) while patients are being enrolled and more frequently if deemed necessary by the PIs or DSMB chairman.

II. DSMB Membership

The DSMB for this trial is the Medical College of Wisconsin Cancer Center DSMB. Potential conflicts of interest that develop during the conduct of this trial should be disclosed to the DSMB chair or Principal Investigator.

III. DSMB Overall Responsibilities

The DSMB will be asked to meet prior to study enrollment, then approximately every 6 months thereafter. When the study is approved and begins enrolling at outside sites, the DSMB is responsible for the overall study monitoring.

- The DSMB will meet twice a year (approximately every 6 months) unless an ad hoc meeting is requested.
- The DSMB will be responsible for overseeing the emerging safety data of the study.
- The DSMB, based on data review and stopping rules, will be responsible for assessing whether there is any basis upon which to recommend modification or premature termination of the study.
- The DSMB will be able to review any of the primary study data, following a specific notification to the institution.
- It is the DSMB responsibility to preserve confidentiality of the results and conclusions.
- The DSMB chairperson is responsible for all DSMB interaction with the principal investigator, study committee and/or institutions.
- The DSMB chairperson is responsible for communications with the study committee through the research study coordinator.
- The DSMB chairperson is responsible for reporting data-driven conclusions and/or decisions to the primary investigator, study statistician and study committee.
- The DSMB chairperson will interact with the study statistician. The study statistician will produce and distribute reports to the DSMB members.
- The study committee and study coordinator are responsible for addressing safety issues identified by the DSMB.

- The study's statistician will address any statistical question raised by the DSMB during the data review, and will perform data analyses according to the DSMB's request.

IV. DSMB Meeting

An initial meeting will be conducted with members of the DSMB. The Principal Investigator will be invited to attend the beginning of the meeting as per the DSMB policy. The DSMB will familiarize themselves with the protocol and the statistical plan, the safety monitoring plan will be approved by all members of the DSMB, and the format and content of the data for review will be agreed upon. After this initial meeting, the DSMB will meet twice a year (approximately every 6 months) to review specific protocol data unless an ad hoc meeting is requested.

V. DSMB Data Review

A. Scheduled Review

- Data for review will be provided by the Principal Investigators, study statistician and study coordinator prior to each DSMB bi-yearly meeting and will be sent no later than 14 days ahead of the meeting.

- Data will include a list of all adverse events, serious adverse events and all DLTs of each enrolled patient, together with demographic summaries of the patient population and other relevant information, in a format that will be agreed upon in the initial meeting.

- Data regarding mortality due to infections will be reviewed for possible increased incidence based on published literature or institutional experience.

- The report will include all data reported up to 30 days prior to the date of the preparation of the above items. Other information as requested by the DSMB will also be provided.

B. Unscheduled Review

- A DSMB committee meeting can be called anytime at the discretion of the DSMB chair and/or by the study PIs' request.

VI. DSMB Reports

The DSMB chair will provide the study coordinator and Principal Investigator with a report including minutes of the meeting and recommendations regarding whether to continue the trial as planned, suggest modifications, suspend the trial or close the trial after review. This report will be dispersed to local and outside center IRBs, as well as to the FDA as part of the IND Annual Review.

VII. Confidentiality

The material contained in each DSMB review is confidential. Until the final study results are public, DSMB members are not to reveal any information, in verbal or written form, to any person outside the committee. Documents containing results should be stored in a locked area, or returned to the study coordinator at the conclusion of each meeting.