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I. Title: A Phase I/II Study Evaluating the Safety and Efficacy of Adding a Single Prophylactic Donor Lymphocyte Infusion (DLI) of Natural Killer Cells Early After Nonmyeloablative, HLA-Haploidentical Hematopoietic Cell Transplantation – A Multi Center Trial

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II. INTRODUCTION

Often the only cure for high-risk hematological malignancies is allogeneic hematopoietic cell transplantation (HCT). However, finding suitable HLA-matched donors continues to be markedly difficult for certain patients, especially those having small family units, backgrounds of high genetic diversity, or those belonging to underrepresented ethnicities. In this regard, HLA-haploidentical family donors have emerged as easily accessible and viable alternative donor sources. In FHCRC Protocol 1667, the use of HLA-haploidentical related donors with nonmyeloablative conditioning and potent immunosuppression has proven to be a well-tolerated, low-toxicity regimen, with a majority of patients engrafting and having a low incidence of severe graft-versus-host disease (GVHD). This is in contrast to many other studies, where graft rejection, GVHD, or infections due to delayed immune reconstitution tend to be excessive after using HLA-haploidentical donors. In particular, the Perugia group has explored the use of HLA-haploidentical donors in the myeloablative setting. Using grafts containing a “mega-dose” of CD34 enriched, extensively T cell depleted stem cells, they observed an excessive mortality of 40% due to infectious complications. The lower rates of non-relapse mortality (NRM) seen in Protocol 1667 may be credited to using a nonmyeloablative preparative regimen, thus reducing toxicity and infectious risks, using bone marrow over mobilized peripheral blood stem cells (PBSC), thus reducing GVHD, and using cyclophosphamide (CY) after HCT, which causes bidirectional deletion of alloreactive T cells responsible for GVHD and graft rejection.

However, while we have overcome this strong immunological barrier to provide both engraftment and low GVHD, 57% of patients are dying by two years after HCT from either early or late relapse despite most having full donor chimerism. This has led to the hypothesis that CY treatment after HCT ultimately contributed to the elimination of important donor T as well a natural killer (NK) cell clones necessary for immune surveillance and tumor kill. In order to preserve the advantage of low GVHD while improving tumor surveillance and control, we now propose two separate interventions: 1) continuing to eliminate alloreactive lymphocyte clones early after HCT with CY, and later 2) augmenting the graft-versus-tumor (GVT) effect with an infusion of purified donor NK cells. Donor lymphocyte infusions (DLI) containing high numbers of CD3+ cells can successfully elicit GVT effects after transplantation, but its use is limited in the early period after HCT due to the risk of developing myelosuppression, seen particularly in marrow-occupying malignancies, and life-threatening GVHD. The add-back of DLI purged of alloreactive T cells has also been studied by many groups seeking to improve immune reconstitution after T-cell depleted, HLA-haploidentical HCT, although this may not be an obvious solution to counteract relapse. Purified NK cell add-back studies have been attempted in the clinical setting and have corroborated the feasibility of NK cell enrichment, its ability to facilitate GVT effects, and have confirmed its low potential to elicit GVHD and other toxicities in the post-transplantation setting. Building upon these promising results, we now propose the use of a single, purified infusion of donor NK cells on day +7 after HLA-haploidentical, nonmyeloablative HCT as pre-emptive, adoptive immunotherapy to prevent relapse. The effects of this donor NK infusion on engraftment kinetics, NK cell phenotype development, killer cell immunoglobulin-like receptor (KIR) expression, and T cell reconstitution will also be monitored closely after HCT.

III. BACKGROUND

A. A Brief Review of Allogeneic HCT Using HLA-Haploidentical Donors

HLA-haploidentical stem cells have been recognized as an important alternative donor source, with multiple groups around the world attempting to improve outcomes using either

Table 1. Selected outcomes of interest from HLA- haploidentical related treatment regimens using myeloablative and non-myeloablative regimens[1]

Institution	*n*=	Preparative Regimen	GVHD ppx	1˚ Graft Failure	Acute GVHD	NRM	Outcomes
MYELOABLATIVE							
FHCRC[2]	269	CY/TBI	CSP±MTX;MTX only; none	8.5%	"mod-severe": 67%	NA (17/269: aplasia)	NA
Royal Marsden	35	CY/TBI or CY/Mel	CSP ±MTX	29%	80%	57%	OS 6 mo-3 yrs: 31%
Milwaukee	48	TBI/CY/AraC/ MP	Ex vivo TCD w/ $T_{10}B_9$ or OKT3	4%	II-IV: 46%	42%	2 yr OS: 21%; 2 yr DFS: 17%
JSHCT	142	TBI/CY or Bu/CY	CSP/MTX (87%) or others (13%)	6.3%	III-IV: 30%	NA	NA
USC	201	TBI/VP16/ AraC/CY/ ATG	CSP, partial ex vivo TCD (w/ $T_{10}B_9$ or OKT3), MP, ATG	2%	II-IV: 13% III-IV: 15%	51%	5 yr OS:19% 5 yr DFS: 18%
Beijing, China	135	Bu/CY/AraC/ MeCCNU/ ATG	CSP/MTX/ MMF+ in vitro TCD w/ ATG	0%	II-IV: 40% III-IV: 16%	22%	2 yr OS: 71% 2 yr DFS: 64%
Perugia	104	TBI/TT/FLU/ATG	Ex vivo TCD via CD34 selection	7%	II-IV: 8%	40%	5 yr EFS: 47% (for pts in remission at HCT); 4% (in rel at HCT
Tubingen	63	TBI or BU-based with FLU/TT/ATG/CY	Ex vivo TCD via CD34 or CD133 selection	17%	II: 7% III-IV: 0%	29%	3 yr DFS: 48% (NHL/ ALL in remission at HCT); 0% (no remission) 60%: NMD
Japan Multicenter Study	135	TBI-based in majority of pts	CSP/FK506/MTX/steroids ± ex vivo TCD via CD34 selection	13%	II-IV: 21%	47%	5 yr DFS: 39% (std-risk pts) 5% (high-risk pts)
Canadian Multicenter Study	11	Mel/TT/FLU/ATG	Ex vivo TCD via CD34 selection	0%	0%	55%	9 mo DFS: 9%; OS: 9%
NON-MYELOABLATIVE							
Duke Univ	49	FLU/CY/ Campath	MMF±CSP + in vivo TCD via Campath	6%	II-IV: 16% III-IV: 8%	10.2%	1 yr OS: 31%
Tubingen/ Dresden	10	FLU/TT/Mel/ OKT3	Ex vivo TCD via anti-CD3/CD19 MAb	0%	II: 60%; III: 0% IV: 10%	30%	OS: 50%
Tubingen	38	FLU/TT/Mel	Ex vivo TCD via anti-CD3/CD19 MAb	17%	II-IV: 27%	2.6%	EFS: 70% (good risk) 20% (poor risk)
MGH	12	CY/Anti-CD2Mab/ Thymic XRT	CSP±ex vivo TCD via CD34 selection and in vivo TCD via anti-CD2 MAb	0%	II-IV: 17%	25%	15-34 mo DFS: 17%; OS: 25%
Osaka Univ	26	FLU/BU/ATG	FK506/MP + in vitro TCD via ATG	4%	II: 20% III-IV: 0%	15%	3 yr EFS: 55% 664day OS: 58%
Tokyo Univ	12	Myelo (n=6): TBI/CY/Campath RI: FLU/BU/ Campath± TBI 4 Gy	CSP/MTX + in vivo TCD via Campath	0%	III-IV: 9%	17%	1 yr OS: 35%
JHU	13	FLU/CY/TBI	CSP/MMF + in vivo TCD via CY	31%	II-IV: 17%	8%	6 mo DFS: 38%, OS: 46%

*mechanism not mentioned; Ara-C: cytarabine; ATG: anti-thymocyte globulin; Bu: busulfan; CSP: cyclosporine; CY: cyclophosphamide; DFS: disease-free survival; FK506: tacrolimus; FLU: fludarabine; IBMTR: International Bone Marrow Transplant Registry; JSHCT: Japan Society for Hematopoietic Cell Transplantation; MeCCNU: methyl-1-(2-Chloroethyl)-3-Cyclohexyl-1-Nitrosourea; Mel: melphalan; MGH: Massachusetts General Hospital; MP: methylprednisolone: MTX: methotrexate; myelo: myeloablative; NA: not available; NMD: non-malignant disease; RI: reduced-intensity; TCD: T cell depletion; TT: thiotepa; USC: Univ of South Carolina; XRT: radiation therapy.

myeloablative or non-myeloablative regimens. The Perugia group is perhaps the most well-known for studying the strategy of using highly T-cell depleted, "mega-dose" CD34-selected PBSC preceded by myeloablative conditioning, thus rendering the pt highly lymphopenic, and no immune suppression is given after transplantation. Virtually all patients engrafted (95%) and none developed GVHD. However, NRM was 40% due to infections, and relapse occurred in 30% of all patients, with a more striking effect in those with ALL (50%) compared to AML (10%)[3].

Multiple centers have attempted to improve on or replicate the Perugia results with mixed findings. A recent review by the group from Duke University[1] compiled published results from centers using myeloablative and non-myeloablative regimens with varying degrees of T cell depletion and post-transplantation immunosuppression for GVHD prophylaxis. The summary of these heterogeneous results can be found in **Table 1**, demonstrating that these institution-dependent treatment plans still have considerable room for improvement. Specifically, GVHD, relapse, and infectious complications are still having a negative impact on overall survival.

B. HLA-Haploidentical Transplantation for Malignant Diseases Using CY After HCT

1. Using CY after HCT

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[4] and Kastan[5] 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[6] 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[7] 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[8] 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 FHCRC. However, it could not be tested in these studies whether the beneficial effect of ablating these proliferating T cells to decrease the incidence of GVHD would have any undue effects in a malignant disease model. Further, work done at the NIH[9] 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.

2. Results of phase II nonmyeloablative clinical trials using HLA-haploidentical donors: the Johns Hopkins University (JHU) and Seattle collaborative experience

Based on a successful Phase I study conducted by O'Donnell and colleagues at JHU (n=13)[10], 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 in Seattle and at Johns Hopkins University in Baltimore[11]. These studies differed by immunosuppression only: the Baltimore regimen utilized 2 doses while the Seattle regimen used 1 dose of CY after HCT

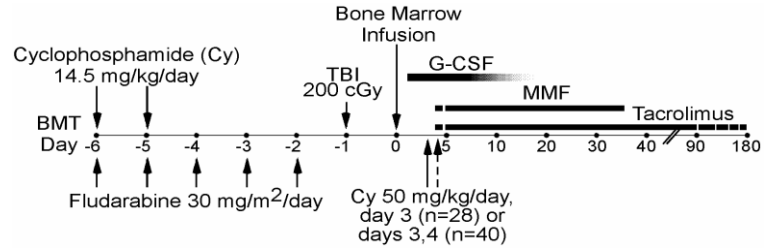

Fig 1. HLA-haploidentical, nonmyeloablative HCT Treatment Plan
(CY=cycophosphamide, FLU=fludarabine, MMF=mycophenolate mofetil, G-CSF= granulcotye colony stimulating factor)

(**Figure 1**). 25% of all patients were from ethnic minorities, in part due to the greater genetic diversity seen in African Americans, and highlighting the lower prevalence of underrepresented populations in bone marrow registries. 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). 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 (**Figure 2**). More notable was the cumulative incidence of grade III GVHD of 6% at 1 year with no grade IV GVHD (**Figure 3**).NRM was 4% at day +100 and 15% at 1 year with infections being the leading causes of NRM (**Table 2**). 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 **Figure 4**, supporting the need for anti-tumor intervention early after HCT.

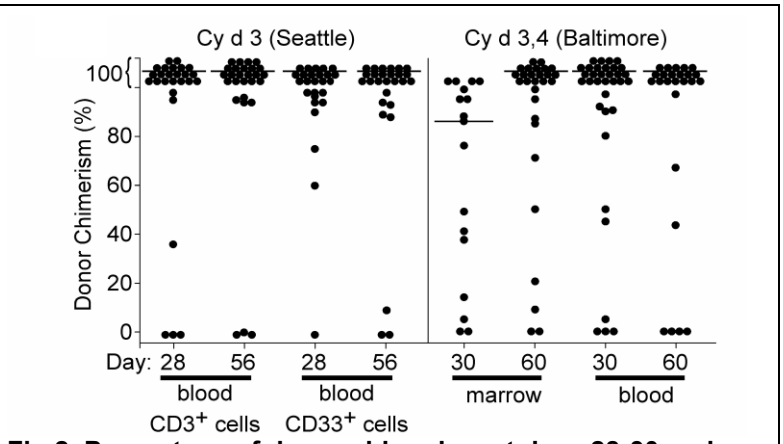

Fig 2. Percentage of donor chimerism at days 28-30 and 56-60 by group.

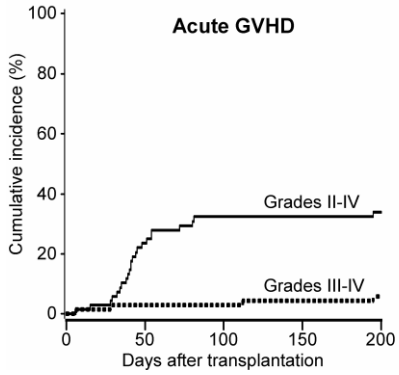

Fig 3. Cumulative incidence of acute GVHD in both groups.

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 GVHD, as is seen with alemtuzumab-based regimens[12], relapse is a considerable cause of mortality. This supports the need for an early, preemptive intervention to facilitate GVT effects after HCT. **Figure 5** graphically shows the degree of lymphodepletion apparent after nonmyeloablative conditioning and the introduction of CY early after HCT in 36 patients who have been treated in Seattle on Protocol 1667.

Table 2. Causes of Death (Relapse and Non-Relapse Mortality) Across 2 Studies

Causes of Death	Seattle (n=28)	Baltimore (n=40)
Relapse	**12**	**19**
GVHD	**1**	**1**
Infection	**2**	**2**
CNS Hemorrhage	**0**	**2**
Unknown	**1**	**1**
Secondary AML	**1**	**0**

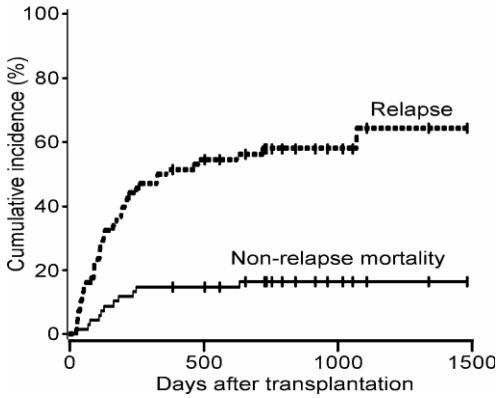

Fig 4. Incidence of Relapse and NRM across both studies

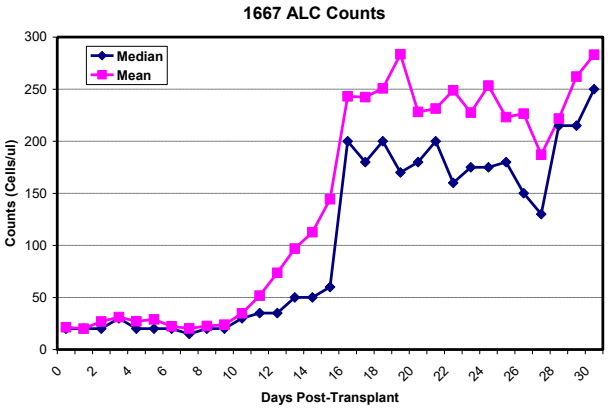

Fig 5. Absolute Lymphocyte Counts (ALC) in 36 patients treated on Protocol 1667 in Seattle

C. Experiences Using Non-Conventional Forms of DLI as Adoptive Immunotherapy after Allogeneic HCT

The infusion of DLI containing high numbers of CD3+ cells can contribute to myelo-suppression, especially after marrow-infiltrative diseases, and life-threatening GVHD after HLA-mismatched HCT[13]. To circumvent these issues, a multi-center group from Italy conducted a Phase I study testing the safety and feasibility of repeated infusions of allogeneic, ex vivo expanded cytokine-induced killer cells (CIK), also known as NKT cells, for patients relapsing after 0-1 allelic HLA-mismatched HCT. Of eleven patients enrolled, none experienced infusional toxicity after CIK infusion, and four developed acute GVHD (≤ grade II). However, six showed no clinical response and died of rapidly progressive disease. Meyer et al attempted prophylactic DLI, depleted of CD8 cells, on days 60 and 120 after T-cell depleted, reduced intensity HLA-matched HCT. Of 8 CD8-depleted DLIs collected, the median CD3-/CD16+CD56+ dose was 128.4 (range, 82.6-444.2)x 10^9. Of 23 patients enrolled, only 11 met criteria for prophylactic DLI due to the persistence of low-grade acute GVHD. Of these 11, 7 developed acute GVHD (I=5, II=1, III=1) and had disease-free survival (DFS) of 68.2% (95% CI, 37.3-99.1) compared to DFS 52.5% (95%CI, 28.5-76.5) for the whole patient population (n=23). Overall, this CD8-depleted DLI was well-tolerated[14].

In the HLA-haploidentical setting, the use of DLI purged of alloreactive T cells has been pursued by several groups. Brenner's group from Baylor University co-cultured donor peripheral blood with recipient EBV-transformed lymphoblastoid cell lines that were generated six weeks earlier. After 72 hours of incubation, co-cultures containing alloreactive T-cells were depleted using anti-CD25 monoclonal antibody, washed, and infused into patients at days 30, 60, and 90 after myeloablative, CD34-selected, T-cell depleted HLA-haploidentical HCT. Of 16 patients given T-cell purged DLI, GVHD occurred in two (grades II and IV) and higher numbers of effector (rather than memory) T cells were generated, thus improving immune reconstitution. However, only five of 16 achieved DFSI[15], indicating only modest GVL effects in this patient population This group has further studied the ability of expressing a suicide gene, inducible caspase 9, into DLI containing very large numbers of allodepleted T cells as a mechanism to allow T cell destruction should adverse events occur, thus adding a measure of protection in DLI-induced GVHD[16]. Additionally, the Perugia group has studied the effect of photodynamic purging of alloreactive T cells as a way to improve on DLI containing

large numbers of CD3+ cells [17]. The latter two studies are in preclinical stages, with no published clinical results to date.

D. NK Cells

1. Biology of NK cells

NK cells are ideal for developing an adoptive immune strategy to decrease relapse after HCT. Due to their MHC-unrestricted ability to kill malignant cells, NK cells can provide tumor surveillance when T cells cannot. Specifically, malignant cells, which often evade the T cell adaptive immune response through down-regulation of MHC class I molecules on their cell surfaces[18,19], can still be effectively targeted by NK cells. This ability, maintained through the NK cell's lack of engagement of its MHC-specific receptors to the target cell, initiates cytolytic activity by suppressing the inhibitory signal that normally protect cells from autoimmunity when the receptor binds to its ligand[20 21]. This phenomenon is referred to as "missing self" recognition, allows for NK cell killing of targets when the ligand is not expressed. However, NK cells also express receptors that, when in contact with its ligand, can generate activating signals based on intracellular signaling through the length of their cytoplasmic tails[22]. This activating signal may be sufficient to engage NK cell activity, despite the presence of concurrent inhibitory signals, and as such reduced expression of MHC Class I is not always necessary to initiate NK cell function [23]. Thus, it is the use of both activating and inhibitory signals that influences the overall NK cell immune response. This quality can be exploited for adoptive immunotherapy in the HLA-haploidentical setting. Furthermore, it is now understood that NK cells can be further split into at least two main categories, those that are $CD56^{bright}$ and those that are $CD56^{dim}$. These different populations perform different functions based on their degree of KIR expression, ADCC activity, cytokine production, and graft-versus-tumor effects [24].

NK cell receptors, which recognize antigens at HLA-A, B, or C and are known as killer immunoglobulin-like receptors (KIR), are inhibitory receptors and are highly polymorphic; they are encoded on chromosome 19 and are clonally distributed within the NK cell repertoire. Those that recognize MHC Class I amongst other ligands are the conserved CD94/natural killer group 2 (NKG2) receptors, encoded on chromosome 12. The differential expression of these two receptors defines the NK cell repertoire. Furthermore, NK cells' ability to home to sites of infection, produce inflammatory cytokines, and kill normal cells infected with parasites, viruses, and bacteria without prior sensitization[25] further illustrates NK cells' importance in maintaining a robust system of defense against both malignant and infected cells. As such, NK cells play a pivotal role in the innate immune response.

2. Effect of immunosuppressive drugs 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, which suppress T cell function, are of concern in NK cells due to their shared lymphocyte ancestry. In the 1980's, it was shown that 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[26]. Further, dogs undergoing non-myeloablative HCT with post-grafting MMF and CSP still have substantially higher lytic activity on in vitro ^{51}Cr release NK functional assays compared to untreated controls[27]. In a rodent model, CSP inhibited T cell but not NK cell responses[28]. 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-g producing NK cells were detected in CSP-treated cultures compared to controls. Mechanistically, CSP works as a calcineurin inhibitor. 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[29]. Thus, it is not expected that the donor NK cell infusion will be adversely affected by ongoing post-grafting immunosuppression.

3. Using highly purified NK cell infusions as adoptive immunotherapy

Further in vitro studies by Aversa et al[30] demonstrated that a human AML NOD/SCID mouse model infused with human alloreactive NK cells could survive until sacrificed at 120 days compared to 3 weeks if either left untreated (i.e., no NK cells) or given non-alloreactive human NK cells. In this mouse model, NK cells also converted mixed into full donor chimerism and did not elicit GVHD even when infused in large numbers. Such observations by the Perugia group suggested that alloreactive NK cells in the donor-versus-host direction are responsible for GVL effects, having a stronger impact on myeloid malignancies with no contribution to GVHD.

NK cells taken from HLA-haploidentical donors have been used for adoptive immunotherapy both in non-transplant and transplant studies. These studies corroborate the ease of NK cell collection and the safety of infusing NK cells into patients. One concern for using NK cells has been the ability to obtain large enough numbers for infusion to provide adequate immune surveillance. Several groups have studied methods of expanding NK cells ex vivo by incubation with either cytokines (i.e., IL-2),[31,32] or Notch ligands[33,34]. However, in the early period after transplantation when abundant cytokines are released[35], NK cells will easily expand and thus this issue is of little concern in this proposed protocol.

Miller and colleagues[36] were the first group to demonstrate the ability of NK cells to expand in vivo and used donor NK cell infusions to treat persistent, refractory, or relapsed disease in a non-transplant setting. NK cells were enriched through the CD3+ depletion of peripheral blood mononuclear cell (PBMC) apheresis products from HLA-haploidentical donors. These cells were incubated with IL-2 overnight and infused into patients who were then given daily IL-2 injections for 14 days. All patients (n=43) were primed with one of three different chemotherapeutic regimens to prevent immediate rejection of donor cells. These regimens differed in either the degree of lymphopenia and/or neutropenia that they elicited. Results demonstrated that those patients experiencing the highest degree of chemotherapy-induced lymphopenia (19/43) before the NK cell infusion had the most robust in vivo expansion of NK cells (as measured by unique RT-PCR primers for donor specific MHC Class I alleles), and of these patients, 5/19 achieved complete remission. IL-2 stimulated donor NK cells in one case persisted for as long as 138 days. This study suggests that the environment into which exogenous NK cells are placed substantially impacts the degree of NK cell expansion, function, and persistence. This need for in vivo NK cell expansion, whether by way of exogenous IL-2 supplementation as demonstrated in Miller's study[36], or by abundant cytokines uniformly released during the early period after HCT[35], appears to be crucial in promoting NK cell survival and proliferation.

Passweg and colleagues[37] developed a pilot study (n=5) evaluating the safety and feasibility of collecting and infusing donor NK cells for mixed/declining chimerism or early relapse after myeloablative, HLA-haploidentical HCT. The Miltenyi system was used for both CD3 depletion and CD56 selection, producing both a highly purified NK cell population (median purity 97.3%) and a

high degree of T cell depletion (median log T cell depletion -3.55). After infusing these enriched NK cells, no patients on this study experienced infusional toxicities, acute or chronic GVHD, or late toxicities. NK cells were infused between 3-26 months after HCT and demonstrated mixed results. Specifically, progressive disease (n=1), persistent mixed donor chimerism (n=2), full donor chimerism (n=1) and graft failure (n=1) were all observed. Although limited by small numbers, this study highlights the need that delayed NK cell infusions given as a last resort in response to clinical necessity may not be early enough to prevent undesired outcomes; preemptive therapy may provide superior and more durable results.

4. The effect of KIR ligand mismatches after HCT

Several studies have examined outcomes after KIR-ligand mismatched HCT, reporting both encouraging[30,38 39] and deleterious[40 41] effects. This ongoing controversy may be explained by differences in the number of residual host and/or donor T cells present within the host hematopoietic microenvironment. Host and donor lymphopenia can be highly variable due to the intensity of conditioning or T cell depletion of the donor allograft, respectively. The function of these residual T cells on NK cell development is actively being investigated by many groups. A retrospective study performed Cooley et al in Minnesota[42] examined the role of T cells in HLA-matched donor grafts and their effect on NK cell development and subsequent clinical outcomes in patients transplanted for CML. Thirty-seven patients received unrelated, unmanipulated marrow while 40 received unrelated, T-cell depleted marrow. Recipient samples collected at day +100 (median +98, range +70 to +130) were evaluated as a single timepoint and compared to donor pre-transplant samples. Findings included: 1) the diminished expression of KIR in patients receiving unmanipulated marrow compared to those receiving T cell depleted marrow (p=0.001); 2) no difference in the increased expression of CD94/NKG2A between the two types of donor sources; 3) the increased expression of interferon gamma-producing NK cells after unmanipulated compared to T cell depleted marrow HCT (p=0.007), despite both groups having the same 4-fold increase in NK cells at day +100 (p=0.26), suggesting improved NK cell lytic activity in unmanipulated marrow recipients; 4) the increased incidence of acute GVHD in those patients having NK cells with higher interferon gamma production; and 5) increased KIR expression is independently predictive of increased survival, having a relative risk of 0.36 (p=0.37). It appears from this study that the interaction between T and NK cells in the early period after HCT is complex, and that residual donor T cells may have a detrimental effect on NK cell function and development.

A study of NK cell receptor maturation has enabled insight into the lineage-specific commitment of CD34+ stem cells into mature, functional NK cells. Cooley et al in Minnesota[43] have been able to induce differentiation of an immature, poorly-functioning NK cell, defined as lacking inhibitory receptors NKG2A-/KIR-/CD56^{dim}, into a productive, effector cell expressing KIR and/or NKG2A receptors. The implication of this finding is that our previous understanding of NK cell alloreactivity may need to be challenged. Specifically, some NK cells lacking inhibitory receptors may actually be developmentally immature cells. After being exposed to a cytokine-rich environment, the cells may mature into NK cells expressing inhibitory receptors. As such, these cells, which may have originally been considered to be of an alloreactive nature due to their lack of expressing inhibitory receptors, may actually promote self-tolerance. Thus, this study provides even more understanding for discrepant clinical results seen from multiple centers regarding KIR alloreactivity effects after HCT.

Given the lack of consensus, we will not use KIR criteria for donor selection but will collect samples for retrospective evaluation in this study.

5. Effect of NK cells on immune reconstitution after HCT

Other studies have evaluated NK cell reconstitution and found unique patterns in the NK cell repertoire after HCT. Shilling et al from Stanford[44] identified three unique patterns of NK cell reconstitution after patients (related =13; unrelated n=5) underwent HLA-matched HCT for leukemia. This group showed that in most cases, it took recipients between 6 months and 3 years to recapitulate the donor NK cell phenotype. The first group (n=8) consisted of those whose KIR expression became that of donor by 1 year after HCT. None of the recipient/donor pairs had coincidental sharing of KIR genotype. The second group (n=5) comprised patients who had reduced KIR expression, but whose CD94/NKG2A expression remained high at 1 year after HCT. CD94/NKG2A expression is considered an earlier phenomenon of NK cell reconstitution before KIR is fully expressed on mature NK cells[45]. Of this group, one recipient/donor pair had coincidental sharing of KIR genotype while another pair, although KIR genotype disparate, had similar NK cell repertoires prior to HCT. However, in three patients from whom 3 year post-transplant samples could be collected, all eventually converted to KIR repertoires similar to that of their donors. The third group (n=5) consisted of patients who developed unique NK cell repertoires which were neither of donor nor of host origin. Interestingly, two recipient/donor pairs had identical KIR genotypes. This idiosyncratic finding is not fully understandable, although the authors make an observation that patients in the third group suffered from major clinical complications after HCT, including acute grade IV GVHD, chronic extensive GVHD, and relapse, although this does not fully explain this finding as patients in other groups also had relapse and GVHD (although low grade and not extensive). In summary, this study showed that in most cases, KIR expression eventually converted to that of donor, although it is not well understood why some converted faster, and why others developed unique NK cell repertoires neither of host nor donor origin.

Nguyen and colleagues in Paris have specifically looked at NK cell reconstitution after HLA-haploidentical HCT and also confirmed that residual T cells in the graft affected the function and repertoire of NK cells after HLA-haploidentical transplantation. In one of their studies[46], 21 patients with advanced malignant hematopoietic disease underwent myeloablative conditioning consisting of 8 Gy TBI (day -5), thiotepa 20 mg/kg/day (day -4), rabbit ATG 5 mg/kg/day (days -3, -2), and cyclophosphamide 60 mg/kg/day (days -3, -2), with no post-transplantation GVHD prophylaxis. Eleven patients received partial (pTCD) (1.4 - 23 CD3+cells/kg) or extensive (eTCD) ($<10^5$ CD3+ cells/kg) T cell depleted, GCSF-mobilized PBSC. Findings demonstrated that in pTCD patients, $CD56^{dim}$ NK cells were found in similar levels in post-transplant recipients as in their donors. These $CD56^{dim}$ cells had higher cytolytic activity than the predominant NK cell found in eTCD patients, which were $CD56^{bright}$ NK cells and considered the more primitive subtype. Interestingly, 7 of 10 patients in the eTCD group died from early relapse (median day +115, range 59-185) despite 8 having a KIR-ligand mismatch in the graft-versus-host direction, while 1 of 11 patients in the pTCD died from relapse with 4 having KIR ligand mismatch in the same direction. This further supports the idea that eliminating donor T cells from the graft predisposes patients to relapse, either by direct T cell effects or indirectly by way of influencing NK cell reconstitution. In a previous study[47], this group also suggested that the overexpression of CD94/NKG2A on immature NK cells early after HLA-haploidentical HCT renders these cells ineffective in killing leukemic blasts.

E. The Effect of NK Cell Engraftment Kinetics Compared to Other Cell Lineages on Outcomes after Allogeneic HCT

Our group has previously analyzed lineage-specific chimerism in 120 patients after non-myeloablative HCT. A statistically-significant improvement in relapse-free survival was noted only after rapid establishment of donor NK chimerism **(Table 3)**[48]. A higher statistically significant association was seen in a recent update incorporating 282 patients. NK cell chimerism on days 14-42 were associated with a lower risk of relapse (p=0.006) and better progression-free survival (p=0.003) in time-dependent analyses, and no other lineages emerged as significant. Higher NK cell chimerism was not associated with higher rates of GVHD (p=0.29), unlike higher T cell chimerism (p=0.007) **(Table 4)**. A day-14 NK cell chimerism level of < 50% was associated with increased risks of graft rejection (p=0.009). The qualitative associations between donor NK cell chimerism and graft rejection, GVHD, relapse or progression-free survival did not change after adjustment for the presence of recipient KIR ligands nor after adjustment for the number of donor inhibitory or activating KIR genes[49].

Table 3. Association between donor chimerism levels and death (any cause) or relapse/progression *% donor chimerism modeled as a continuous linear variable; hazard ratio presented as increase in donor chimerism of 20%

	Hazard Ratio*	95% CI	P
Granulocytes	**0.89**	**0.72-1.10**	**0.28**
T cells	**0.89**	**0.72-1.06**	**0.19**
Monocytes	**0.89**	**0.72-1.08**	**0.21**
NK cells	**0.79**	**0.64-0.96**	**0.02**

Table 4. Increasing T cell rather than NK cell chimerism is significantly associated with higher rates of acute GVHD

	# Patients with Acute GVHD / # Pts at risk (%)	
% donor chimerism on day 14	**T-cells**	**NK cells**
0-50	**21/46 (46%)**	**10/20 (50%)**
51-75	**32/62 (52%)**	**20/37 (54%)**
76-90	**30/41 (73%)**	**31/53 (58%)**
91-100	**9/12 (75%)**	**15/23 (65%)**
Trend	***P*=.007**	***P*=0.29**

Savani et al from the NIH looked at the recovery of NK cells in 54 patients receiving T-cell depleted, HLA-identical sibling myeloablative HCTs between 1993 and 2005. In a univariate analysis, transplants involving donor KIR genes 2DL5A, 2DS1, 3DS1 (found in 46% of patients) and higher numbers of inhibitory donor KIR had better transplant outcomes, and by multivariate analysis, day 30 NK cell counts >150/μL had less relapse (HR 18.3, p=0.039), acute GVHD (HR 3.2, p=0.03), NRM (HR 11.4, p=0.03) and improved overall survival (HR 11.4, p=0.03)[50].

A group from Korea also examined the effect of non-CD34+ cells and their effect on early engraftment. In 69 patients who received either myeloablative (Bu/CY, n=51) or reduced-intensity (FLU-based, n=18) regimens, higher numbers of transplanted cytotoxic (CD8+) and NK (CD56+) cells predicted earlier engraftment with better HCT outcomes than higher numbers of CD34+ cells[51]. When comparing engraftment kinetics, they found that neutrophil engraftment was highly correlated with higher numbers of transplanted NK cells (p<0.001), T cells (p<0.001), MNC (p=0.005), and B cell (p=0.032) lineages, but not with numbers of transplanted CD34+ cells (p=0.442). They also found that it was higher numbers of transplanted NK and T cells that influenced immune reconstitution. Specifically, a higher transplant dose of NK cells was strongly associated with CD3+ T cell (p=0.092) and CD56+ NK cell reconstitution between 3 months and 1 year after HCT. This further supports the choice of using an enriched and highly purified NK cell infusion rather than CD34-selected PBSC as adoptive immunotherapy.

F. Donor Issues

HLA-haploidentical, related marrow donors undergo both a marrow harvest for the initial transplant, and then 6-9 days later, a 10-12L leukapheresis (adjusted for donor age/weight and equal to 2-3 total blood volume (TBV) for both pediatric and adult donors, which is considered Standard Practice

for donor apheresis) to collect lymphocytes for NK cell enrichment. If the selected donor is unwilling and/or unable to undergo both procedures or storage of autologous blood prior to marrow harvest, then another HLA-haploidentical related donor must be found. If the donor is an adult, at least 18 years old, and peripheral venous access is not adequate then a central venous catheter (CVC) will need to be temporarily placed. In such a situation, the risks and benefits of the CVC will need to be weighed carefully in order for the donor to make an informed decision. Children less than 12 years of age are excluded from being a donor in this study. Children greater than or equal to 12 years of age will be considered as a donor for this study if the following two criteria are met: 1) they must be able to give assent in the presence of the parent (or legal guardian) and an Attending physician who is not a member of the recipient's care team, 2) they must be able to undergo apheresis using peripheral IV access; CVC placement is not an option for children. For donors weighing >40 kg, one unit of autologous blood will be collected on two separate occasions, at approximately 3-4 weeks and 2-3 weeks prior to the marrow harvest. There must be at least one week between the collection of the units, and the second autologous unit must be collected no less than two weeks before the marrow harvest. If the donor weighs <40 kg, only 10 mLs/kg autologous blood will be collected on each occasion for storage. Donors will start oral iron supplementation after collection of the first unit of autologous blood. For the marrow harvest, no more than 20 mL/kg donor weight of marrow will be collected, based on National Marrow Donor Program (NMDP) standards. Donors will likely develop anemia due to the marrow harvest. To ensure an adequate hematocrit to safely undergo apheresis, the previously-collected autologous blood will be infused into the donor in the recovery room and prior to discharge after the marrow harvest. Since autologous blood is not routinely given to an otherwise healthy donor in the absence of severe anemia post-operatively, the donors should be counseled about the unique indication in this setting and the general risks of blood transfusion. There is no expectation that platelet counts will be low following a marrow harvest, thus the patient should not need a platelet transfusion prior to the apheresis procedure. One week after the marrow harvest, donors will undergo apheresis. Mononuclear cells (MNC) collected from the apheresis procedure will be stored overnight at 1-6 C for Miltenyi CliniMACS processing the following day.

G. Enrichment of NK Cells

Donor MNC will undergo both a CD3 depletion followed by a CD56 selection with the Miltenyi CliniMACS system using Good Tissue Practices. The entire volume of processed cells, with a safety limit of $<10^4$/kg of CD3+/CD56- cells, will be infused. This 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[52]. After the CD56 enrichment, the cells will be spun down out of the Miltenyi buffer and resuspended in cell therapy laboratory (CTL) standard infusion media (Normosol-R plus 1% Human Serum Albumin) and stored at room temperature until ready to be infused. All reagents and material used during processing and final formulation have been verified to be sterile and endotoxin free. Should the CD3+ fraction of the product exceed this limit, then the volume of the product will be reduced accordingly while maintaining the NK cell dose at ± 20% of the proposed dose level cohort. 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 from the microbiology lab, 3) the number of residual CD3+ cells is $<10^4$/kg. The infusion of the NK cell product will take place on the same day as the processing. ***If patients experience hyperacute GVHD actively requiring <u>systemic</u> therapy (other than prophylactic tacrolimus and MMF) or are receiving greater than or equal to 0.25 mg/kg equivalent prednisone dose for any reason within one day of the NK cell infusion, we will not infuse the***

Table 5. Results from a non-G-CSF stimulated normal volunteer using the Miltenyi CLINIMACS system to perform a CD3 depletion / CD56 selection

	TNC	CD3+	CD3+/CD56+	CD56+
Start	9.16×10^9	4.3×10^9	3.5×10^8	9.2×10^8
CD3 depleted	2.87×10^9	5.7×10^6	3.9×10^6	7.9×10^8
CD56 enriched	4.94×10^8	1.1×10^5	1.8×10^6	4.6×10^8

donor NK cells. If already collected, NK cells may be cryopreserved and may be used off-study at a later timepoint. Additionally, steroids are to be 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, document clearly in clinical notes. Patients will remain on study. Unused NK cells from which donor identifiers have been removed may also be used for other IRB-approved research studies, which is described in the Donor Consent.

Three full-scale collections using volunteer donors have been conducted at our Center using CD3 depletion and CD56 selection via the Miltenyi system. Results of one collection from a non-G-CSF stimulated donor are shown **(Table 5)**, demonstrating an enriched, pure NK cell population (94%) with a 4.6 log T cell depletion (courtesy of S. Heimfeld).

IV. RATIONALE FOR STUDY

We propose that an infusion of donor NK cells on day +7 after nonmyeloablative, HLA-haploidentical HCT will not only provide a temporary boost of mature NK cells to treat low-level disease and hasten engraftment, but will also favorably impact early immune reconstitution after HCT. We further propose that this infusion of donor NK cells will influence the development of particular NK cell and T cell subtypes which will provide both immediate and long-term tumor surveillance, infectious monitoring, and sustained engraftment.

V. OBJECTIVES

A. Phase I Objective

Identification of the maximal feasible dose of NK cells that can be infused one week after nonmyeloablative, HLA-haploidentical HCT

B. Phase II Objectives

Once the maximal feasible dose has been identified, accrual will be limited to the cohort containing this cell dose to determine:

1. Incidence of relapse
2. Incidence of grades III-IV acute GVHD
3. Incidence of non-relapse mortality

VI. PATIENT SELECTION

A. Inclusion Criteria

1. Patients with the following hematologic malignancies will be permitted although other diagnoses can be considered if approved by PCC and the principal investigators:
   - Aggressive non-Hodgkin lymphomas (NHL) and other histologies such as Diffuse Large B cell (DLBC) NHL– a) not eligible for autologous HCT, b) not eligible for high-dose HCT, c) after failed autologous HCT, or d) be part of a tandem auto-allo approach for high risk patients
   - Mantle Cell NHL – must be beyond first CR
   - Low grade NHL– with < 6 month duration of CR between courses of conventional therapy
   - CLL – must have either 1) failed to meet NCI Working Group criteria for complete or partial response after therapy with a regimen containing FLU (or another nucleoside analog, e.g. 2-CDA, pentostatin) or experience disease relapse within 12 months after completing therapy with a regimen containing FLU (or another nucleoside analog); 2) failed FLU-CY-Rituximab (FCR) combination chemotherapy at any time point; or 3) have “17p deletion” cytogenetic abnormality and relapsed at any time point after any initial chemotherapy.

- Hodgkin Lymphoma – must have received and a) failed frontline therapy, b) not be eligible for autologous HCT, or c) or be part of a tandem auto-allo approach for high risk patients.
- Multiple Myeloma or Plasma Cell Leukemia – must have received more than one line of prior chemotherapy. Consolidation of chemotherapy by autografting prior to nonmyeloablative HCT is permitted.
- Acute Myeloid Leukemia (AML)–Must have < 5% marrow blasts at the time of HCT
- Acute Lymphocytic Leukemia (ALL)-Must have <5% marrow blasts at the time of HCT.
- Chronic Myeloid Leukemia (CML) – accepted if they are beyond CP1 and if they have received previous myelosuppressive chemotherapy or HCT, and have <5% marrow blasts at time of transplant
- Myelodysplasia (MDS)/Myeloproliferative Syndrome (MPS) – (>int-1 per IPSS) after ≥ 1 prior cycle of induction chemotherapy. Must have <5% marrow blasts at time of transplant.
- Waldenstrom's Macroglobulinemia – must have failed 2 courses of therapy.

2. No age restrictions
3. Patients must be expected to have disease controlled for at least 60 days after HCT
4. Patients for whom HLA-matched unrelated donor search could not be initiated or completed due to insurance reasons, concerns of rapidly progressive disease, and/or discretion of attending physician are eligible for this protocol

B. Exclusion Criteria

1. Patients with available HLA-matched related donors
2. Patients eligible for a curative autologous HCT
3. Significant organ dysfunction that would prevent compliance with conditioning, GVHD prophylaxis, or would severely limit the probability of survival:
   a. 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. DLCO <40% TLC <40%, FEV1 <40% and/or receiving supplementary continuous oxygen. The FHCRC study PI must approve enrollment of all patients with pulmonary nodules.
   c. Liver function abnormalities: 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
4. HIV seropositive patients
5. Patients with poorly controlled hypertension despite multiple antihypertensive medications.
6. 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
7. Fertile males who are unwilling to use contraceptive techniques during and for the twelve months following treatment
8. Patients with active non-hematologic malignancies (except non-melanoma skin cancers) or those with non-hematologic malignancies (except non-melanoma skin cancers) who have been rendered with no evidence of disease, but have a greater than 20% chance of having disease recurrence within five years
   This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy
9. Active infectious disease concerns

10. Karnofsky performance score <60 Lansky performance score <60
11. Life expectancy severely limited by diseases other than malignancy
12. Patients with a diagnosis of CMML.
13. CNS involvement with disease refractory to intrathecal chemotherapy.
14. Patients with AML, MDS, ALL, or CML must not have presence of circulating leukemic blasts detected by standard pathology.
15. Patients with aggressive lymphomas (such as DLBC) must not have bulky, rapidly progressive disease immediately prior to HCT.
16. Patients who have received a prior allogeneic HCT must have no active GVHD requiring immunosuppressive therapy for at least 21 days prior to start of conditioning

VII. DONOR SELECTION

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 the only allowed hematopoietic stem cell source
3. Haploidentical donor selection will be based on standard institutional criteria, otherwise no specific prioritization will be made amongst the suitable available donors. Donors will not be selected based on KIR status.

B. Exclusion Criteria

1. Children less than 12 years of age.
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 years of age who have inadequate peripheral vein access to safely undergo apheresis.
4. Donors unable or unwilling to undergo marrow harvest for the initial HCT, storage of autologous blood prior to marrow harvest or apheresis one week after marrow harvest
5. Donors who are not expected to meet the minimum target dose of marrow cells (1×10^8 nucleated cells/kg recipient IBW) for the initial HCT. *The average nucleated cell content of harvested marrow is 22×10^6 nucleated cells/mL or 220×10^8 nucleated cells/Liter.*
6. HIV-positive donors
7. Donors who are cross-match positive with recipient

VIII. INFORMED CONSENT

Patients will be referred here for consideration of a marrow transplant. 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. 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 should include graft rejection, GVHD, infections, and death. With the donor, discussion about the potential risks of marrow donation, autologous infusion of packed red blood cells, 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 FHCRC'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). A summary of the conference will be documented for the medical record detailing what was covered.

IX. PROTOCOL REGISTRATION

Eligible patients will be identified by the Clinical Coordinator's Office, who will register the patients with the Registration Office (206-667-4728) between 8:30 am and 4:00 pm, Monday through Friday. After hours, the Registration Office can be reached by paging (206) 995-7437.

X. PLAN OF TREATMENT

A. 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 outpatient as medically appropriate. 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. See **Figure 6** for details.

	Days											
	−6	−5	−4	−3	−2	−1	0	+3	+4	+7	+40	+84	+180
Conditioning													
CY 14.5 mg/kg (no MESNA)	●	●											
FLU 30 mg/m^2/day	●	●	●	●	●								
TBI 200 cGy						●							
HCT (Marrow only acceptable stem cell source)							0						
Post-Transplantation								+3	+4	+7	+40	+84	+180
CY 50 mg/kg								●					
MESNA (dosed 100% CY dose)								●					
MMF (refer to XII.F for dosing)									Start	→	Taper*	Stop*	
Tacrolimus (refer to XII.E for dosing)									Start	→	→	Taper*	Stop*
G-CSF 5μg/kg/day									Start^				
Donor NK Cell Infusionº										●			

* Based on GVHD Status
^Continue G-CSF until ANC>500 x 3 days

º **Dose Level 1: NK cell dose 2.5 x 10^6/kg**
Dose Level 2: NK cell dose 5 x 10^6/kg

Fig 6. HLA-Haploidentical, Nonmyeloablative HCT with Donor NK Cell Infusion Treatment Plan Conditioning consists of CY 29 mg/kg, FLU 150 mg/m^2, and 200 cGy TBI. Bone marrow is infused on Day 0. G-CSF begins day+4 after marrow infusion and continues until engraftment is seen. Post-transplantation immunosuppression consists of CY (day +3) MMF, and tacrolimus. Both MMF and tacrolimus must begin at least 24 hours after the CY infusion in order to prevent blunting of the alloreactive response needed for effective CY targeting of proliferating lymphocytes. A single infusion of donor NK cells at a dose of either **2.5 or 5 x 10^6 CD56+/CD3- cells per kg** is given on Day +7 (or day of NK cell infusion). Collections in the Phase II portion of the study that do not achieve the NK target dose will be infused and patients will be evaluable for the Phase II outcomes

1. Conditioning Regimen

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

a. Fludarabine

i. Fludarabine dose is based on estimated creatinine clearance

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 iothalamate study
(Age <10): Iothalamate study is recommended

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

Table 6: 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 >100% of the Ideal Body Weight.
If the Ideal Body Weight (IBW) > Actual Body Weight, then Actual Body Weight will be used.

c. Total Body Irradiation

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

2. Marrow Infusion

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.

See **Appendix A** for ABO Incompatibility guidelines.

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 Day +3, CY will be given as a single dose of 50 mg/kg IV
Use Adjusted Body Weight; if Ideal Body Weight is greater than Actual Body Weight, use Actual Body Weight. Post-transplant CY will be given within 48-72 hours of marrow 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. Mycophenolate Mofetil

i. Starting on Day +4 (24-36 hours after last dose of CY), MMF will be given orally at a dose of 15 mg/kg based on adjusted body weight every 8 hours.
MMF will be tapered after day +40 (adapted dose-reduction to be discontinued by day+84) if there is no evidence of GVHD.

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 +4. 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.

c. Tacrolimus

i. Starting on Day +4 (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 twice weekly thereafter and the dose adjusted accordingly to maintain a level of 5-15 ng/ml. Tacrolimus will be tapered after day +84 (adapted dose-reduction to be discontinued by day +180) if there is no evidence of GVHD.

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) Whole blood trough levels of tacrolimus (i.e., just prior to the next dose) should be obtained on Day +8 and then 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.

Table 7: Medications that Affect Tacrolimus 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.	

4. Growth Factor Support

Patients will receive G-CSF at 5 μg/kg/day IV or SC starting at day +4 and continuing until the ANC >500/uL for 3 days. Refer to **Section X.A.3.b.iii** for information regarding prolonged neutropenia.

5. Donor NK Cell Infusion

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

Mononuclear cells (MNCs) will be collected from the COBE apheresis machine on Day +6. If it is not feasible to collect cells on Day +6, MNCs can be collected between Days +6 to +9 (although the preference is that they be collected on Day +6). For Seattle patients, the apheresis product will be transported to the SCCA Cell Therapy Laboratory (Main Lab: 288-1200; Product Development Specialist: Lori Jones, 288-8278), where MNCs will be stored overnight at 1-10°C for processing the following day. Other study sites will follow their internal procedures.

The morning after collection, the MNCs will undergo a CD3 depletion followed by CD56 selection using the Miltenyi CliniMACS system using GTP standard procedures. Details regarding the enrichment and purification process can be found in **Section III.G.** The NK cell product will be stored at room temperature until ready for infusion.

If patients experience hyperacute GVHD actively requiring systemic therapy (other than prophylactic tacrolimus and MMF) or, for any reason, are receiving greater than or equal to 0.25 mg/kg equivalent prednisone dose (within one day of the NK cell infusion), we will not infuse the donor NK cells. If already collected, donor NK cells may be cryopreserved and may be used off-study at a later timepoint. Unused NK cells from which donor identifiers have been removed may also be used for other IRB-approved research studies, which is described in the Donor Consent.

b. Characterization of NK Cell Product and Byproducts

i. NK Cell Product

a) Cellular Phenotype

An aliquot of the enriched product will undergo cellular phenotyping.

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 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) the 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 (within a 20% window) to be enrolled at the particular dose level. 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 two dose levels on this study are:
Dose Level 1 -- NK cell dose of 2.5 x 10^6 (± 20%) CD56+/CD3- cells per kg.
Dose Level 2 -- NK cell dose of 5 x 10^6 (+ 20%) CD56+/CD3- cells per kg.

Once the Phase I study is completed and the maximal feasible dose has been identified, subsequent patients will be enrolled on the Phase II study at the maximal feasible dose. Collections in the Phase II portion of the study that do not achieve the NK target dose will be infused and patients will be evaluable for the Phase II outcomes.

b) The enriched NK cell product may also be phenotyped for expressed NK cell receptors, or activating and inhibitory KIR.

ii. Byproducts

In addition, the two byproduct fractions containing the CD3+ cells and CD56- cells may also be phenotyped for cellular characterization and cryopreserved per batch record worksheet. Since these fractions will not be infused into the patient, it may be important to document which cell types are being discarded from the final infusion product. KIR analyses will not be done on the byproducts as there should be minute numbers of NK cells remaining.

c. NK Cell Product Repository

A cell repository will be created from the NK cell product for use in future studies; this includes additional unused NK cells. Cells will be cryopreserved using controlled rate freezing and stored in liquid nitrogen freezers.

d. Infusion of Donor NK Cells

Donor NK Cells will be infused into the recipient the same day as the product is processed as long as three release criteria are met (refer to **X.A.5.b.i**). Due to the length of time it will take for processing, the NK infusion will likely take place in the late afternoon or evening the day after the product is collected. The infusion of these cells will follow established standard procedures for donor lymphocyte infusions, including administration of appropriate pre-medications, having anaphylaxis medications immediately available, and having close monitoring during and after the 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 SCCA outpatient clinic. All pediatric patients will receive the NK cell product as inpatients.

Standard guidelines for cellular product infusion currently in place at the SCCA require a 2 hour monitoring period to observe any potential adverse events from the end of infusion. However, in order to comply with FDA requirements, the Phase I portion of the study will require a 4 hour window of observation after donor NK cell infusion. All attempts will be made to ensure that the infusion will take place as an outpatient. However, if the 4 hour observation window cannot be completed during normal hours of the SCCA outpatient clinic. Adult patients will need to be admitted for their NK cell infusions. Once the Phase I portion of the study has been completed, we may consider decreasing the monitoring period to 2 hours post-infusion for the Phase II portion of the study if no severe infusional reaction (grade IV toxicity using the Adapted Common Toxicity Criteria) has been observed.

Potential side effects of the NK cell infusion include anaphylaxis, acute GVHD, nausea, vomiting, infection, diarrhea, and marrow suppression, in particular prolonged neutropenia. These side effects are considered rare. One extremely rare potential side effect may be the development of human-anti-mouse antibodies (HAMA) from the Miltenyi beads. To maximize effectiveness of the NK cells, steroids are to be avoided within 1 day BEFORE until 2 days AFTER receiving the NK infusion unless there is medical necessity. Patients receiving >0.25 mg/kg equivalent prednisone dose within 1 day prior to the NK cell infusion will not receive NK cells. **Patients not receiving NK cells (for any reason) will be taken off-study**. However, patients who must be given steroids for medical necessity within 2 days AFTER receiving the NK cell infusion will continue to remain on study.

DONOR RESEARCH SAMPLES
Peripheral blood and bone marrow aspirate will be obtained at different timepoints for Immune Studies. Please see Appendix M for details.

B. DONOR TREATMENT PLAN

Table 8. Donor Treatment Plan

	Days			
	Week 4 to 3 Pre	**Week 3 to 2 Pre**	**Day 0**	**+6**
Autologous RBC Collection (1 or 2 Units*)	●	●		
Oral iron supplementation x 7 days	●	●		
Marrow Harvest			●	
Infusion of Autologous RBC (1-2 Units)			●	●
COBE Apheresis**				●

*If only one unit is needed, the collection can occur 1 to 4 weeks prior to bone marrow harvest
** COBE Apheresis may take place between days +6 and +9 to allow for scheduling flexibility. Preferred to be performed 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. These guidelines are also reflected within FHCRC Standard Practice Guidelines for Donor Selection. Donors will be undergoing both marrow harvest for stem cell collection 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

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. 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 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

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). 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 ideal body weight as specified above. 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 (see **Appendix A**). Following the marrow harvest, donors will be transfused autologous blood during post-anesthesia recovery to replenish blood loss from the harvest bleeding into tissues, and to minimize anemia prior to planned apheresis at day 6 to 9 post-harvest.

4. Peripheral Blood Mononuclear Cell (MNC) Apheresis

A CBC will be drawn one day prior to planned MNC apheresis. 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. The apheresis is preferred to take place on day +6 (can occur between days +6 and +9 to facilitate scheduling). A total of 10 to 12 liters of donor blood volume will be processed by MNC 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 CTL facility for overnight storage and will be processed the following day, as described in **Section X.A.5.a.**.

XI. DONOR AND RECIPIENT EVALUATIONS

A. HLA-Typing of Patient and Potential Donors

1. As broad a range of potential related donors as possible should be typed. Included are parents, siblings, eligible children, and cousins.
2. Serotyping (HLA-A, B, C) and DNA typing (HLA-A, B, C, DRB1, DQB1) of patient and donor will be performed.
3. Leukocyte and/or florescence activated cell sorter cross match between the patient and donor will be done. Recipient must have a negative cytotoxic cross-match to donor lymphocytes, otherwise an alternative HLA-haploidentical donor must be identified.
4. KIR genotype will not be used in determining donor selection.

5. Blood samples for HLA-typing should be sent to: Clinical Immunogenetics Lab for HLA-typing (heparinized green top tube, 10 cc), 206-667-7700; SCCA G7-200.

B. Donor Evaluations

Per Standard Practice Guidelines, to also include:

1. Complete history and physical examination prior to marrow harvest and interval history and a nursing check the day after harvest

2. Lab tests:
   a) **CBC with differential including platelet count:** per Institutional Guidelines with marrow harvest
   b) **ABO Rh blood typing.** If the recipient has antibodies against red cell antigens of the donor, the titers must be determined. Appropriate RBC depletion of the marrow allograft will need to be done per standard procedures.
   c) **Infectious disease testing** per standard practice, as part of the initial evaluation and within 1 week of donor apheresis (see **X.B.1**)
   d) **Donor Research Tests**
   Peripheral blood may be collected from the donor on 1-2 separate occasions during the pre-transplant work-up in order to perform the Immune Studies (See Appendix M)

3. Donor Chimerism Reference
   As a pre-transplant reference for subsequent determination of donor chimerism 10 mLs of heparinized (green top) peripheral blood from the donor will be drawn. For FHCRC patients: Send to Clinical Immunogenetics Lab (206-667-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. Label the sample with “Protocol 2230.”

C. Recipient Evaluations

1. Pre-transplant Evaluations

a) See Institutional Standard Practice Guidelines for Standard Pre-Transplant Evaluation Guidelines.

b) Peripheral blood and bone marrow aspirate may be collected from the recipient during the pre-transplant work-up. If the patient is lymphopenic, an additional peripheral blood draw may be required to yield adequate cells for the research test. See Appendix M for additional research specimen requests

c) **See Table 9, 10, 11 and, 12 for disease specific pre-transplant evaluation.**

Table 9: Disease-Specific Pre-Transplant Evaluations for Ph (-) ALL, Ph (+) ALL, CML

Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected **within 21** days of treatment. See Tables 13 and 14 for post-transplant evaluations and table 15 for additional lab instructions.

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	
PCR for bcr/abl, p.210 breakpoint - **see comment*	Clinical	**CML only- reflective testing for FHCRC patients only*
PCR for bcr/abl, p.190 and p.210 breakpoints - **see comment*	Clinical	**Ph (+) ALL only- reflective testing for FHCRC patients only*
Bone marrow biopsy		
Pathology- **see comment*	Clinical	**CML only*
Peripheral Blood		
Storage for chimerism analysis	Clinical	
PCR for bcr/abl, p.210 breakpoint- **see comment*	Clinical	**CML only*

Table 10: Disease-Specific Pre-Transplant Evaluations for AML and MDS/MPD

Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected within **21 days** of treatment. See Tables 13 and 14 for post-transplant evaluations and table 15 for additional lab instructions.

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	
Bone marrow biopsy		
Pathology- **see comment*	Clinical	**MDS/MPD only*
Peripheral Blood		
Storage for chimerism analysis	Clinical	

Table 11: Disease-Specific Pre-Transplant Evaluations for CLL, HL, NHL

Note: All bone marrow aspirates and biopsies are **bilateral** and must be collected within **30 days** of treatment. See Tables 13 and 14 for post-transplant evaluations and table 15 for additional lab instructions.

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry- *see comment	Clinical	*No HL
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	
PCR t(11:14) - *see comment	Clinical	*Mantle Cell NHL only
PCR t(14:18) - *see comment	Clinical	*Follicular NHL only
Bone marrow biopsy		
Pathology- *see comment	Clinical	*HL – only if history of BM involvement
Peripheral Blood		
Storage for chimerism analysis	Clinical	
Quantitative Ig levels	Clinical	
β-2 microglobulin	Clinical	
LDH	Clinical	
ZAP – 70 by flow cytometry- *see comment	Clinical	*CLL only– for patients not in CR
Imaging		
CT of chest, abdomen, pelvis (neck if indicated)	Clinical	

Table 12: Disease-Specific Pre-Transplant Evaluations for MM and Waldenstrom's Macroglobulinemia

Note: All bone marrow aspirates and biopsies are **bilateral** **see comment* and must be collected within **30 days** of treatment. See Tables 13 and 14 for post-transplant evaluations and table 15 for additional lab instructions.

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate **see comment*		**Unilateral bone marrow aspirate is acceptable for MM*
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	
Bone marrow biopsy **see comment*		**Unilateral bone marrow biopsy is acceptable for MM*
Pathology	Clinical	
Peripheral Blood		
Storage for chimerism analysis	Clinical	
SPEP/IFIX	Clinical	
Quantitative Ig levels	Clinical	
β-2 microglobulin	Clinical	
Cryoglobulins, c-reactive protein, serum viscosity - **see comment*	Clinical	**Serum viscosity only for patients with >3gm/dL IgM monoclonal protein or >4gm/dL IgA or IgG protein*
Urine		
UPEP/IFIX	Clinical	
Protein / creatinine clearance	Clinical	
Imaging		
MRI – **see comment*	Clinical	**MM only. A PET scan is acceptable.*
Skeletal survey – **see comment*	Clinical	**MM only*
CT of chest, abdomen, pelvis (neck if indicated) – **see comment*	Clinical	**Waldenstrom's Macroglobulinemia only*

2. Post-transplant Evaluations

See Table 13 for disease specific post-transplant evaluation on Day +28, 56, 84, etc. This is a recommended evaluation schedule.

Patients should be assessed for the need of bisphosphonates and IVIG monitoring and replacement therapy per Institutional Guidelines

See Standard Practice Guidelines for Evaluation Guidelines Prior to Departure from FHCRC System. GVHD evaluation guidelines are as follows:

- History and physical exam
- Skin biopsy
- Schirmer's tear test
- Pulmonary function test
- Oral exam
- CXR
- Dietician assessment
- Gynecological departure assessment (adult female)

See Appendix M for additional research requests

Table 13: Post-Transplant Evaluation

This is a recommended evaluation schedule.
See Tables 9 - 12 for pre-transplant evaluations. Additional lab instructions in Table 15.

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
Ph (-) ALL	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets (CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
Ph (+) ALL	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical		x	x	x	x	x	x	x
	FISH for bcr/abl and other clonal abnormalities	Clinical		x	x	x	x	x	x	x
	PCR for bcr/abl, p.190 and p.210 breakpoints	Clinical		x	x	x	x	x	x	x
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	PCR for bcr-abl, p.190 and p.210 breakpoints	Clinical	*If Bone Marrow not obtained, reflexive testing for FHCRC patients only	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
AML	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
MDS/ MPD	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical	*For pts. with evidence or history of myelofibrosis	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
CML	**BM aspirate** *see biopsy ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical		x	x	x	x	x	x	x
	FISH for bcr-abl and other clonal abnormalities	Clinical		x	x	x	x	x	x	x
	PCR for bcr-abl, and p.210 breakpoint	Clinical	*If abnormal pre-transplant, Reflexive testing for FHCRC patients only	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	PCR for bcr-abl, and p.210 breakpoint	Clinical	*If abnormal pre-transplant AND bone marrow not obtained, Reflexive testing for FHCRC patients only	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
CLL	**BM aspirate** *see biopsy ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical				x	x	x	x	x
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	Flow cytometry	Clinical	*If peripheral blood involvement pre-transplant AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical				x		x		
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual
				**28	56	84	180	1	1.5	x 5 years
HL - No history of BM involvement	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical				x		x		
	Cytogenetics	Clinical	*If abnormal pre-transplant			*See comment		*See comment		
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant			*See comment		*See comment		
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical				x		x		
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual
				**28	56	84	180	1	1.5	x 5 years
HL - History of BM involvement	**BM aspirate** *see biopsy ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical				x	x	x	x	x
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical				x		x		
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
NHL – No history of BM involvement *see separate section for additional PCR on Mantle Cell and Follicular NHLs in suspected CR	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical				x		x		
	Flow cytometry	Clinical				x		x		
	Cytogenetics	Clinical	*If abnormal pre-transplant			*See comment		*See comment		
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	Flow cytometry	Clinical	* If peripheral blood involvement pre-transplant AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 Microglobulin	Clinical				x				
	LDH	Clinical				x	x	x	x	x
	Imaging									
	CT of chest, abdomen, pelvis (neck if indicated)	Clinical	*If abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
NHL -- History of BM involvement *see separate section for additional PCR on Mantle Cell and Follicular NHLs in suspected CR	**BM aspirate** *see biopsy ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical				x	x	x	x	x
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	Flow cytometry	Clinical	*If peripheral blood involvement pre-transplant AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical				x		x		
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
Mantle Cell NHL *in suspected CR*	**BM aspirate** **in addition to complete NHL restaging* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	PCR for t(11:14)	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood **in addition to complete NHL restaging*									
	PCR for t(11:14)	Clinical	*If abnormal pre-transplant AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
Follicular Cell NHL *in suspected CR*	**BM aspirate** **in addition to complete NHL restaging* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	PCR for t(14:18)	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood **in addition to complete NHL restaging*									
	PCR for t(14:18)	Clinical	*If abnormal pre-transplant AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
MM *Omit SPEP/IFIX and UPEP/IFIX for non-secretory MM*	**BM aspirate** * If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for chrom. 13 (and other clonal) abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	SPEP and IFIX	Clinical				x	x	x	x	x
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical				x	x	x	x	x
	Cryoglobulins, C-reactive protein, viscosity	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment		*See comment
	Urine									
	Protein/creatinine clearance	Clinical				x	x	x	x	x
	UPEP and IFIX	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	Imaging									
	Complete skeletal survey	Clinical						x		x
	Skeletal MRI	Clinical						x		x
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				**28	56	84	180	1	1.5	
Waldenstrom's Macro-globulinemia *Omit SPEP/IFIX and UPEP/IFIX for non-secretory Waldenstrom's Macro-globulinemia*	**BM aspirate** * If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for chrom. 13 (and other clonal) abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism	Clinical	**Needed on Day 7, 14, 28, 56, 84, 180, and 1 year	**See table 14 for peripheral blood chimerisms						
	T and B cell subsets(CD3, CD4, CD8, B and NK cells)	Clinical				x		x		
	SPEP and IFIX	Clinical				x	x	x	x	x
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical					x	x	x	x
	Cryoglobulins, C-reactive protein, viscosity	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment		*See comment
	Urine									
	Protein/ creatinine clearance	Clinical				x	x	x	x	x
	UPEP and IFIX	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Table 14: Post-Transplant Peripheral Blood Chimerism Evaluation

This is a recommended evaluation schedule. See Tables 9 - 12 for pre-transplant evaluations, table 13 for post-transplant evaluations. Additional lab instructions in Table 15.

Disease	Test	Clinical/ Research	Comment	Days						1 Year
				7	14	28	56	84	180	
All Diseases	Chimerism (CD3+)	Clinical	*If donor NK cell infusion is occurring on Day +7, obtain chimerism sample pre-NK cell infusion	*See comment	x	x	x	x	x	x
	Chimerism (CD33+)	Clinical	*If donor NK cell infusion is occurring on Day +7, obtain chimerism sample pre-NK cell infusion	*See comment	x	x	x	x	x	x
	Chimerism (NK CD56+)	Clinical	*If donor NK cell infusion is occurring on Day +7, obtain chimerism sample pre-NK cell infusion	*See comment	x	x	x	x	x	x

Table 15: Additional Lab Instructions

Note: All bone marrow tests are done on aspirate unless specifically identified as biopsy. All instructions apply to both pre- and post-transplant evaluations unless identified otherwise.
Off-site providers may use local facilities for the tests.

Volumes represent desired amounts.

Specimen / Test	Type	Instructions	Lab Name	Contact Information
Bone marrow				
Chimerism	Clinical	1-3mL bone marrow in green-top tube	Clinical Immunogenetics Lab	Seattle Cancer Care Alliance (206) 288-7700
Pathology (*aspirate*)	Clinical	2mL bone marrow in EDTA/ formalin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Pathology (*biopsy*)	Clinical	1cm bone marrow in formalin **OR** mounted in paraffin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Flow Cytometry	Clinical	2mL bone marrow in green-top tube	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
Cytogenetics	Clinical	3mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
FISH	Clinical	2mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
PCR for bcr-abl and p190 and/or p210	Clinical	3mL bone marrow in lavender-top tube Label "protocol 2230"	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
PCR for t(11:14) or t(14:18)	Clinical	2mL bone marrow in lavender-top tube	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
Peripheral blood				
Chimerism (CD3+), (CD33+) NK(CD56+)	Clinical	10mL blood in green-top tube for Flow sorting, then to CIL	UW Hematopathology Lab, routed to Clinical Immunogenetics Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
Flow Cytometry	Clinical	10mL blood in green-top tube	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
T and B cell	Clinical	1mL blood in lavender-top	UW Hematopathology Lab	Seattle Cancer Care Alliance

Specimen / Test	Type	Instructions	Lab Name	Contact Information
subsets(CD3, CD4, CD8, B and NK cells)				(206) 288-7060
SPEP/IFIX	Clinical	3mL blood in red-top tube	UW Department of Laboratory Medicine	University of Washington (800) 713-5198
Quantitative Ig Levels	Clinical	3mL blood in red-top tube	SCCA Alliance Lab	Seattle Cancer Care Alliance (206) 288-2057
β-2 Microglobulin	Clinical	3mL blood in red-top tube	UW Department of Laboratory Medicine	University of Washington (800) 713-5198
LDH	Clinical	3mL blood in red-top tube	SCCA Alliance Lab	Seattle Cancer Care Alliance (206) 288-2057
PCR for bcr-abl and p190 and/or p210	Clinical	7mL blood in lavender-top tube Label "protocol 2230"	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
PCR for t(11:14) or t(14:18)	Clinical	5mL blood in lavender-top tube	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
ZAP – 70 by Flow cytometry (*pre-transplant only*)	Clinical	5mL blood in green-top tube	UW Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060

*See Standard Practice Guidelines for **T and B cell subsets***

Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants) for chimerism analysis.

XII. Drugs, Irradiation and Marrow 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 iphosphamide). 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 (50-100ml) for injection and will be administered by IV infusion over 30 minutes or longer.
   Fludarabine will be administered by IV infusion over 30 minutes in a dose of 30 mg/m^2/day on days -6 to -2.

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 doses of TBI (200-300 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 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. 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 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 X.A.3.c** for information about administration and dosage adjustments.

F. 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

1. Precautions: 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 marrow transplantation, 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 X.A.3.b**.

XIII. 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 Standard Practice Guidelines. Recommendations are listed in **Appendix B** of the protocol.

Highlights include:
1. Sulfamethoxazole/trimethoprim should be started pre-transplant, discontinued on day -2, and reinstituted at Day +30 only if the ANC >500 for three consecutive days; use alternative prophylaxis if ANC <500 by Day+30.
2. Acyclovir should be provided to all transplant patients except those having both negative HSV and VZV serostatus. For duration, refer to chart in **Appendix B**
3. Fluconazole should be given until Day +75

XIV. GRAFT-VERSUS-HOST DISEASE

A. Diagnosis and Treatment
1. Refer to **Appendices C and D** for diagnosis and to Standard Practice Guidelines for latest treatment recommendations for acute and chronic GVHD, respectively
2. Prophylaxis with daily Bactrim and acyclovir 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 25% within the first 100 days after HCT. Details of the rule are described in Section XVI.

XV. EVALUATION AND ENDPOINT DEFINITIONS

A. Study Description
This is a Phase I/II study using nonmyeloablative conditioning (FLU, CY, TBI), HLA-haploidentical marrow as the stem cell source, potent post-transplantation immunosuppression (CY, MMF, tacrolimus), followed by a single NK cell infusion early after HCT.

B. Definition of endpoints
1. Non-relapse Mortality (NRM):
   a. Definition: Death in any patient for whom there has not been a diagnosis of relapse or disease progression.
2. Relapse:
   a. Definition: Presence of malignant cells in marrow (>5% blasts by morphology), peripheral blood (circulating blasts), or extramedullary sites (enlarging lymphadenopathy, soft tissue masses) not evident at the time of HCT. Presence of new or recurrent cytogenetic abnormalities in patients who have previously cleared this abnormality.
   b. Evaluation: Testing for recurrent malignancy will be performed using disease-specific, established testing methods, including but not limited to: marrow and peripheral blood morphology, immunophenotyping, cytogenetics, and molecular assays; peripheral blood counts and tumor markers; radiological evaluation (CT, MRI, PET scans); and biopsy-proven disease.

3. Progression:
   Definition: Advancement of malignancy after HCT despite previous achievement of stable disease (**Table 16**).

4. Acute GVHD:
   a. Definition: Acute GVHD will be defined by the criteria outlined in **Appendix C.**
   b. Evaluation: GVHD will be assessed by a by a single clinician using the criteria in **Appendix C.** 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.
5. Chronic GVHD:
   a. Definition: Chronic GVHD is defined by criteria outlined in **Appendix D.**
   b. Evaluation: Chronic GVHD endpoints will include:
      i. Extent (limited or clinical extensive) of GVHD
      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

Table 16: Definitions of Disease Progression	
Multiple Myeloma or Plasma Cell Leukemia	• **Increasing bone pain or** • **Increase in serum/urine monoclonal protein by 25% or** • **Increase in circulating or bone marrow blasts by 25%**
CLL, NHL, Hodgkin Lymphoma	• **New sites of lymphadenopathy or** • **Increase of ≥ 25% in lymph node size (assessed by CT scan)** • **Blood or marrow involvement with clonal B cells (lymphoma)** • **Increase of ≥ 25% marrow involvement or** • **Increase of ≥ 25% blood involvement of lymphocytes > 50x10^3/µl) with clonal B-cells (CLL)**
CML	• **Inability to control platelet or granulocyte counts** • **Increase in baseline number of metaphases demonstrating the Ph+ chromosome by 25%** • **Any other new cytogenetic abnormality** • **Transformation to accelerated phase or blast crisis**
MDS, AML	• **Aberrant myeloblasts may clear only slowly from the marrow after non-myeloablative HCT. Thus, the presence of residual (<5%) MDS or AMLcells early post-HCT cannot in and of itself be considered evidence for relapse. However, if subsequent marrows show an increase in abnormal cells >5%, this will be considered progressive disease.**

6. Engraftment:
   a. Time to 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. *increasing 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. *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.

XVI. STATISTICAL ANALYSIS

This is a phase I/II study with an initial dose escalation for the targeted NK cell dose, followed by a single cohort phase II at the identified safe dose.

If a patient initially enrolls on this study but is unable to receive a donor NK cell infusion for any reason, the patient will not be included in the statistical analyses for DLT criteria, graft failure, GVHD, relapse, or NRM and they will be considered off-study.

Reasons for not receiving a donor NK cell infusion include, but are not limited to:
1) Patient being actively treated for hyperacute GVHD requiring additional systemic immunosuppressive therapy (other than prophylactic tacrolimus and MMF)
2) Patient receiving >0.25 mg/kg equivalent prednisone dose prior within one day of donor NK cell infusion;
3) Any patient medical concerns or issues that prohibit infusion of the NK cell product
4) Any donor medical concerns or issues that prohibit collection of the NK cell product

Phase I

Two possible targeted doses of NK cells are defined: $2.5x10^6$ cells/kg and $5x10^6$ cells/kg. Three patients will be initially entered at the lower dose level. If none of the 3 experience DLT (as defined below) within 28 days of the donor NK cell infusion, then escalation to the higher dose level will occur. If 1 of the 3 patients experiences DLT then an additional 3 patients will be enrolled at the lower dose level, and if no additional DLTs are observed then escalation to the higher dose may occur. If a 2nd DLT is observed at the lower dose level in any number of patients, then the study will be suspended pending review by our DSMB and consideration of a protocol amendment to establish a lower dose level.

At the higher dose level, the first 6 patients enrolled will be considered part of 'phase I'. If no more than 1 DLT is observed among these 6, then 'phase II' enrollment will commence at this dose level and continue for an additional 29 patients. If a 2nd DLT is observed in the first 6 patients, then the lower dose level will be considered the phase II dose and the additional phase II enrollment of 29 patients will commence at that dose level, provided that at least 6 patients have been enrolled with no more than 1 DLT.

During the phase I enrollment period, patients may be enrolled at the current dose level pending completion of the 28 day observation period for the final patient required to permit dose escalation, provided that at least 2 patients at the current dose level have completed the 28 day observation period without DLT.

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:

1) Grade IV infusional toxicity (based on the "Adapted Common Toxicity Criteria" or CTC)

2) Grade IV regimen-related toxicity (based on Adapted CTC);
3) Grade IV acute GVHD
4) Non-relapse mortality

Phase II

The phase II efficacy evaluation will target a total enrollment of 35 patients: the 6 patients enrolled during phase I plus the additional 29 patients enrolled during phase II. All patients receiving NK cell infusions during phase II will be counted, even if the infused cell dose does not meet the targeted dose established in phase I. The sample size is chosen to provide adequate power to evaluate a possible reduction in the 1-year relapse rate from the historical reference rate of 55% observed in previous studies. With 35 patients, we have approximately 80% power to detect a 20% reduction in the 1-year relapse rate, at a 1-sided 0.10 level of significance. This power calculation is intended only as an approximate point of reference. 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.

Stopping rules will be imposed for:

- Day 200 NRM with a threshold rate of 25%
- Day 100 grade III/IV acute GVHD, with a threshold rate of 25%, or
- Day 100 graft failure, with a threshold rate of 10%.

The limits for NRM and GVHD are somewhat higher than the rates observed in the previous haploidentical donor studies, but we are allowing for the possibility that a decrease in early relapse might lead to a modest increase in these adverse endpoints yet still yield a better overall outcome. The study will stop at any time that there is reasonable evidence that the true rate of these events exceeds the threshold rate. Reasonable evidence will be taken to mean that the lower bound of a 1-sided 80% confidence interval exceeds the threshold. Operationally, the stopping rules would be triggered by the number of patients experiencing the event in question as outlined below:

- Day 200 NRM (25% threshold): 5/10, 8/20, or10/30 patients
- Day 100 grade III/IV acute GVHD (25% threshold): 5/10, 8/20, or10/30 patients
- Day 100 graft failure (10% threshold): 3/10, 4/20, or 5/30 patients

These stopping rules will be evaluated at least every 10 patients. Both the patients and events observed among the 6 patients enrolled during phase I will 'count' for purposes of evaluating the stopping rules. The operating characteristics of these rules are in **Table 17** below.

True rate of day 200 NRM or day 100 GVHD	Probability of stopping[1]	Average N at stopping[1]	True rate of graft failure	Probability of stopping[1]	Average N at stopping[1]
0.3	55%	25	.15	61%	23
0.35	74%	20	.20	83%	18
0.4	89%	16	.25	94%	14
0.45	96%	13	.30	99%	11

[1] estimated from 10,000 Monte Carlo simulations

Enrollment may continue pending evaluation of these endpoints at the 10 patient benchmarks; however, the outcome of subsequently enrolled patients may not override the stopping rule if triggered by patients currently enrolled.
Other endpoints will include evaluation of rejection, overall survival, chronic extensive GVHD, the contribution of KIR ligand alloreactivity to relapse risk, and the effects of a donor NK cell infusion on post-transplant immune reconstitution.

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

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

The principal investigators (PI) will monitor this study, with oversight by a Data Safety and Monitoring Board (DSMB), the Protocol and Data Monitoring Committee (PDMC) and the Institutional Review Board (IRB). 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.

Serious adverse events are reported to the trial coordinator, the study nurse or directly to the PI within 72 hours of the event during the initial 28 days after NK infusion. An official report of an adverse event is faxed to the coordinating center within seven days during the initial 28 days and up to day 180 post HCT. The adverse event report is reviewed by PI. If the adverse event meets the FHCRC criteria for reporting, then an official signed report is submitted to the FHCRC Institutional Review Office (IRO). All deaths, regardless of the cause, are reported to the IRB. This protocol has a DSMB responsible for monitoring patient safety on this clinical trial. The DSMB meets twice a year and all outcome data is reviewed including all adverse events reported to the FHCRC IRO. The DSMB confirms that the trial has not met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMB is submitted to the FHCRC IRB as well as the trial coordinators/local PIs of this protocol. Furthermore, the FHCRC also has a PDMC that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual IRB review and approval is also required.

With respect to safety, patients are monitored for the development of GVHD, myelosuppression, infections and rejection. All patients, regardless of diagnosis, will be considered in the safety analysis. GVHD events will be closely monitored and severity of GVHD graded. Formal stopping rules for GVHD grades III-IV and graft failure before day 100, and NRM before day 200 are provided.

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 FHCRC Trial Coordinator. At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes in the medical record. This documentation is extracted by the study nurse within 140 days +/- after HCT via chart review and collection of copies of source documents and entered into a hard copy or electronic Case Report Form (CRF). The PI reviews the official CRF and primary source documents. After the CRFs are verified, they are signed by the PI. Thus, multiple health care providers provide independent observations and participate in monitoring 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 (fellow or physician assistant), floor or outpatient nurse and the PI or other attending clinician involved with the patient averting 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 on an ad hoc basis as necessary to review the primary data and render a decision.

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

The adverse event reporting will follow the FHCRC Guidelines for serious adverse event (SAE) reporting (**Appendix E**). These guidelines detail the expedited reporting requirements and definitions of particular events. All SAEs that meet expedited reporting criteria are reported to the IRO within 10 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 IRO within 10 calendar days. The PI reviews all SAEs and annual reports at the time of submission. For patients being cared for at the FHCRC, 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 at the time of annual renewal and at the Mixed Chimerism Meeting. The PI for a study is responsible for this reporting and the IRO assures adverse event reporting on an annual basis. The PI in the annual application for grant continuation 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. 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.** At FHCRC, 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 an NCI-funded clinical trial is reported to the NCI 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 IND holder and the Principal Investigator. The IND holder and the principal investigator must report all serious and unexpected adverse events to the IRB, the FDA and the Miltenyi Biotec, Inc. Any temporary or permanent suspension, as determined by the PI, IRB, or PDMC, of this clinical research trial will be reported to the NCI 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 within15 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 serious adverse events occurring during this study, whether or not attributed to study drug, will be included in the IND holder's annual IND report to FDA.

D. Plans for assuring data accuracy and protocol compliance

This protocol has a DSMB that is responsible for reviewing protocol data and safety endpoints. The DSMB meets on a twice-yearly basis, reviews a report of appropriate endpoint data, and compiles a report that is submitted to the IRB, PDMC Chairman, and Protocol Office.

At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations in the medical record. This documentation is extracted by one of the study nurses at 100 days after HCT via chart review and entered into an electronic Case Report Form (CRF). The study nurses also continue to follow patients after day 100, review source documentation, and complete CRFs at 6 month and then yearly intervals per protocol. The CRFs are printed directly from the database, and the PI reviews the CRFs and the primary source documents for data accuracy. When the CRFs are verified, the PI signs them, and the data are incorporated into a central database by the data manager. Thus, multiple health care providers provide independent observations and participate in assessments on this trial. The study has dedicated nurses who at a minimum follow patients to confirm eligibility; reporting of adverse events; reporting of events, which are part of the safety-monitoring plan, and protocol adherence. The PI, data coordinators, and research nurses are responsible for review and maintenance of all patient records to ensure data integrity and protocol adherence.

XVIII. RECORDS

Clinical records will be maintained as confidentially as possible. Data will be collected maintained by Clinical Statistics. The investigator 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. They are maintained by the FHCRC data collection staff, which is supervised by an A.R.T. Access is restricted to personnel authorized by the Division of Clinical Research. Information gathered from this study regarding patient outcomes and adverse events will be made available to the Federal Drug Administration and to Miltenyi Biotec, Inc. All precautions to maintain confidentiality of medical records will be taken.

XIX. PROJECTED TARGET ACCRUAL

Table 18. Projected Accrual based on ethnicity and racial category

TARGETED / PLANNED ENROLLMENT	**Number of Subjects**		
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	0	1	1
Not Hispanic or Latino	14	20	34
Ethnic Category Total of All Subjects*	14	21	35
RACIAL CATEGORIES			
American Indian / Alaska Native	1	0	0
Asian	0	2	1
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	2	1
White	12	17	33
Racial Categories: Total of All Subjects*	14	21	35

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APPENDICES

page

APPENDIX A

ABO INCOMPATIBILITY GUIDELINES

Red Blood Cell - Incompatibility (Major):
Occasional patients may have antibodies directed against red blood cell antigens found on the donor's cells. These are generally ABO or Rh antigens, although incompatibility with other red cell antigens identified by donor-recipient crossmatch may occur. If the recipient shows an anti-donor RBC antibody titer of ≥ 1:32, the RBC content of the marrow product must be reduced to ≤ 20mL in order to avoid a hemolytic transfusion reaction when the product is infused. Incompatible marrow products ≥ 600 mL will be RBC depleted by processing through an apheresis instrument and recovering marrow mononuclear cells (MNC) containing ≤ 20mL of residual RBC. Incompatible marrow products < 600 mL will undergo RBC depletion by the starch sedimentation method. *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*
Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse red cell depleted marrow products within 2 hours of depletion.
Expected Results: Red blood cell depleted marrow products will contain < 20ml residual red blood cells and ≥ 80% recovered marrow MNC.

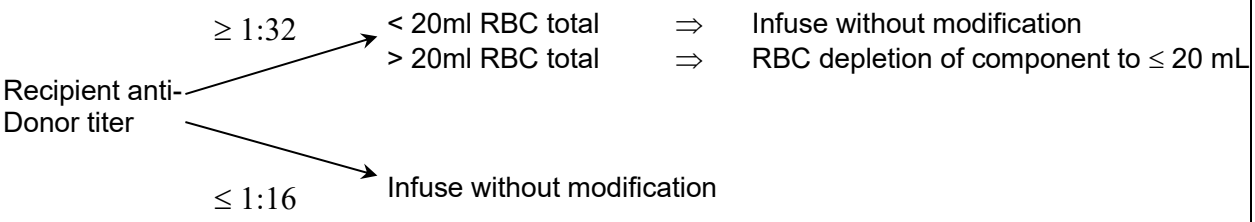
Red Blood Cell - Incompatibility (Minor):
Occasional donors may have antibodies directed against red blood cell antigens (ABO, Rh, or other antigen system) found on the recipient's cells. The risk of hemolysis of recipient red cells immediately after transplant may occur if the donor antibody titer is ≥ 1:256 and the volume of infused plasma (for recipient ≥ 20 kg) is > 200mL. If the donor antibody titer is ≥ 1:256 the marrow product must be plasma reduced to a final volume of ≤ 200 mL (for recipient ≥ 20 kg). For recipients < 20 kg, see the Standard Practice Manual for guidelines regarding final plasma-reduced marrow volumes (see flowsheet below). Due to the high number of lymphocytes in the marrow inoculum, recipients of minor ABO mismatched products may also be at risk for a delayed type of hemolysis that can be severe. *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*
Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse plasma-depleted PBMC within 2 hours of depletion.
Expected Results: The plasma depletion should not affect the nucleated cell recovery.

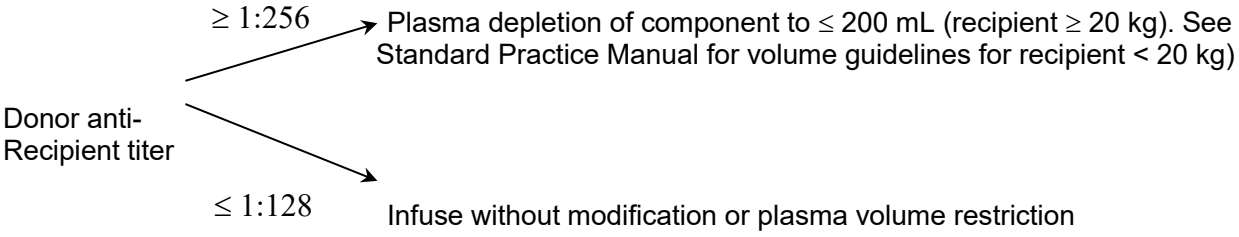
Red Blood Cell – Bidirectional Incompatibility:
Patients undergoing transplants for bidirectional RBC incompatibility should be managed according to both algorithms shown below. Most red cell depletion techniques also deplete plasma from the marrow component with no additional cell loss. *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

2230.00
Post transplant blood component support will be according to Standard Practice Guidelines.

MAJOR ABO INCOMPATIBLE



MINOR ABO INCOMPATIBLE



APPENDIX B

INFECTIOUS DISEASE GUIDELINES

Herpes Simplex and Varicella Zoster Virus Prevention and Treatment

Herpes Simplex and Varicella Zoster Virus

CMV Prevention: Surveillance and Preemptive Therapy

cmvprevention.pdf

CMV Disease: Diagnosis and Treatment

cmvdiseasetreatment.pdf

Antifungal Therapy Guidelines

antifungal_therapy.pdf

Pneumonia / Pneumocystis Jiroveci Prophylaxis

pneumocystisjiroveci

Antibiotic Prophylaxis for Encapsulated Bacteria in Allogeneic Patients with Chronic GvHD Requiring Immunosuppressive Therapy

Antibiotic Prophylaxis for Encap Bacteria

Vaccinations

Vaccinations

Foscarnet

foscarnet.pdf

APPENDIX C
GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE[a]

Severity of Individual Organ Involvement (stage)

Skin	+1	a maculopapular eruption involving less than 25% of the body surface
	+2	a maculopapular eruption involving 25-50% of the body surface
	+3	generalized erythroderma
	+4	generalized erythroderma with bullous formation and often with desquamation
Liver	+1	bilirubin (2.0-3.0 mg/100 ml)
	+2	bilirubin (3-5.9 mg/100 ml)
	+3	bilirubin (6-14.9 mg/100 ml)
	+4	bilirubin > 15 mg/100 ml
Gut		Diarrhea is graded +1 to +4 in severity. 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		
	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	+2	>1,000 ml of stool/day* (> 15ml of stool/kg/day)†
	+3	>1,500 ml of stool/day* (> 20ml of stool/kg/day)†
	+4	2,000 ml of stool/day* (≥ 25ml of stool/kg/day)†

*In the absence of infectious/medical cause
†For pediatric patients

Severity of GVHD (grade)

Grade I	+1 to +2 skin rash No gut or liver involvement
Grade II	+1 to +3 skin rash +1 gastrointestinal involvement and/or +1 liver involvement
Grade III	+2 to +4 gastrointestinal involvement and/or +2 to +4 liver involvement with or without a rash
Grade IV	Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death

[a] From "Graft-vs-host disease" Sullivan, Keith M. *Hematopoietic Cell Transplantation* Ed: D. Thomas, K. Blume, S. Forman, Blackwell Sciences; 1999, pages 518-519

APPENDIX D

EVALUATION OF CHRONIC GRAFT-VERSUS-HOST DISEASE[a]

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 $\geq$ 3x upper limit of normal, AST or ALT $\geq$4x upper limit of normal or total serum bilirubin $\geq$ 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

2230.00
Laboratory testing and diagnostic indicators of chronic GVHD[a]

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

[a] 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 E

STUDY COORDINATOR'S MANUAL

I. Introduction

The mixed chimerism protocols have been opened to multiple sites to increase the referral base and accrual. Because of this expansion of collaborators, the data collection procedures are being revised. The 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 FHCRC IRB and then will be distributed to sites that wish to participate for their 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 consent forms to the FHCRC. 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 FHCRC Principal Investigator. The FHCRC 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 the FHCRC will identify eligible patients. Registration will include completion of the eligibility checklist/demographic form. This form will be faxed (206-667-5378) prior to treatment initiation. Patients should be registered prior to treatment initiation for valid registration

IV. Reporting Adverse Events

The following guidelines are the minimum serious adverse event (SAE) reporting guidelines for Category 1 and 2 studies conducted at the Fred Hutchinson Cancer Research Center.

2230.00
Expedited Reporting Requirements

All unexpected and serious adverse events which may be due to study treatment or intervention must be reported to the FHCRC Institutional Review Office as soon as possible but within at least 10 calendar days of the investigator learning of the event.

Definitions

Adverse Event - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related to the medicinal product.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction. Study toxicities are graded using the adapted NCI Common Toxicity Criteria v.4.0 (where appropriate use the criteria for transplant patients.) All Grade 4 (life-threatening) toxicities occurring between the start of any protocol intervention and day 180 that meet expedited reporting requirements must be reported as soon as possible but within at least 10 calendar days of the investigator learning of the event to the IRO and 72 hours to the IND holder.

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:

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

Hospitalization, in general, will not be considered a serious adverse event as approximately half of evaluable MRD patients AND the majority of evaluable URD patients receiving nonmyeloablative transplants were hospitalized. Hospitalization will be considered a serious adverse event if it fulfills the criteria for a serious and unexpected adverse event as described above.

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

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

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

The FHCRC 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 10 calendar days.

It is the responsibility of the FHCRC 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.

FHCRC is acting as the Coordinating Center for this multi-institutional study, and it is the responsibility of the FHCRC Principal Investigator (or designee) to complete the FHCRC Serious Adverse Event Report for 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 FHCRC Investigator and the IND holder within 72 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 faxed comments to the FHCRC Trial Coordinator at **(206) 667-5378.** This will be followed by detailed written report (See **Appendix I**) within 3 working days. The report must include the date and time of onset, severity and duration of the event, the relationship to the study, the treatment given and eventual outcome. Follow-up information to a SAE report must be submitted as soon as the relevant information is available.

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 (**Appendix F**). 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

Case report forms must be completed for all patients registered onto the protocols and submitted to the FHCRC data coordinating center. The first case report form (day 28) is due on day 50. For

outside centers a Staging Form must accompany the form with the patient staging at registration, day 28, day 56, day 84 and day 100. Staging forms should also be completed with each Follow Up Form completed on day 180, 1 year, 1.5 years, 2 years, 3 years, and yearly thereafter. For Outside Centers, 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, FHCRC 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 FHCRC 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 reapproval 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 Evens 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 F

CORE CASE REPORT FORM

Acrobat Document

APPENDIX G

PROTOCOL 2230 PATIENT DEMOGRAPHICS AND REGISTRATION FORM

Please Fax this completed form to (206)-667-5378 for patient registration.

Questions regarding eligibility should go to Brenda Sandmaier, M.D., 206 667 4961.

UPN: _____

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**

NK CELL DOSE LEVEL ☐ Dose Level 1 -- NK cell dose of 2.5 x 10^6 (± 20%) CD56+/CD3- cells per kg.

☐ Dose Level 2 -- NK cell dose of 5 x 10^6 (± 20%) CD56+/CD3- cells per kg.

Creatinine Clearance:_____ **date:**_____

Fludarabine Dose: _____

Signature of Principal Investigator: _____ **date:**_____

2230.00

Protocol 2230 Eligibility

One of the following criteria questions (1-6) must be marked "Yes" for the patient to enter on 2230. (The following diseases will be permitted although other diagnoses can be considered if approved by PCC and the principal investigator.)

*Patients must *be not eligible for conventional autologous HCT*. Patients for whom HLA-matched unrelated donor search could not be initiated or completed due to insurance reasons, concerns of rapidly progressive disease, and/or discretion of attending physician are eligible for this protocol. Patients must be expected to have disease controlled for at least 60 days after HCT.

1) Yes ☐ No ☐ **Aggressive NHLs and Other Histologies Such as Diffuse large B cell (DLBC) NHL**– a) not eligible for autologous HCT, b) not eligible for high-dose HCT, c) after failed autologous HCT, or d) be part of a tandem auto-allo approach for high risk patients
Low grade NHL– with < 6 month duration of CR between courses of conventional therapy. **Mantle Cell NHL** -must be beyond first CR. **CLL** – must have either 1) failed to meet NCI Working Group criteria for complete or partial response after therapy with a regimen containing FLU (or another nucleoside analog, e.g. 2-CDA, pentostatin) or experience disease relapse within 12 months after completing therapy with a regimen containing FLU (or another nucleoside analog); 2) failed FLU-CY-Rituximab (FCR) combination chemotherapy at any time point; or 3) have "17p deletion" cytogenetic abnormality and relapsed at any time point after any initial chemotherapy. **HL** – Must have received and a) failed frontline therapy, b) not be eligible for autologous HCT, or c) or be part of a tandem auto-allo approach for high risk patients.

2) Yes ☐ No ☐ **Multiple myeloma or Plasma Cell Leukemia** – must have received more than one line of prior chemotherapy. Consolidation of chemotherapy by autografting prior to nonmyeloablative HCT is permitted.

3) Yes ☐ No ☐ **AML/ALL** – must have < 5% marrow blasts at the time of HCT

4) Yes ☐ No ☐ **CML** – Patients will be accepted if they are beyond CP1 if they have received previous myelosuppressive therapy or HCT, < 5% marrow blasts at the time of transplant

5) Yes ☐ No ☐ **MDS/MPD** - (>int-1 per IPSS) after ≥ 1 prior cycle of induction chemotherapy. Must have <5% marrow blasts at time of transplant.

6) Yes ☐ No ☐ **Waldenstrom's Macroglobulinemia –** must have failed 2 courses of therapy.

Each of the following questions (7-20) must be marked "No" Or "NA" for the patient to enroll on 2230.

7) Yes ☐ No ☐ Patient is eligible for a curative autologous transplant.

8) Yes ☐ No ☐ NA ☐ Patient received a prior allogeneic HCT and has active GVHD requiring immunosuppressive therapy for at least 21 days prior to start of conditioning

9) Yes ☐ No ☐ NA ☐ Patient has available HLA-matched related donors

10) Yes ☐ No ☐ Patient has significant organ dysfunction that would prevent compliance with conditioning, GVHD prophylaxis, or would severely limit the probability of survival:

a. 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.
PI Signature: Date: / / .

b. DLCO <40% TLC <40%, FEV1 <40% and/or receiving supplementary continuous oxygen. *The FHCRC study PI must approve enrollment of all patients with pulmonary nodules.*
PI Signature: Date: / / .

c. Liver function abnormalities: 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.

11) Yes ☐ No ☐ Patient is HIV seropositive

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

13) 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.

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

15) Yes ☐ No ☐ Patients with active non-hematologic malignancies (except non-melanoma skin cancers) or those with non-hematologic malignancies (except non-melanoma skin cancers) who have been rendered with no evidence of disease, but have a greater than 20% chance of having disease recurrence within five years
This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy

16) Yes ☐ No ☐ Patient has active CNS involvement with disease refractory to intrathecal chemotherapy.

17) Yes ☐ No ☐ Patient has a Karnofsky score <60 / Lansky score <60

18) Yes ☐ No ☐ Patient has active infectious disease concerns.

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

20) Yes ☐ No ☐ NA ☐ Patient has AML, MDS, ALL, or CML and has presence of circulating leukemic blasts detected by standard pathology.

21) Yes ☐ No ☐ NA ☐ Patient has aggressive lymphomas (such as DLBC) and has bulky, rapidly progressive disease immediately prior to HCT.

22) Yes ☐ No ☐ NA ☐ Patients with a diagnosis of CMML.

2230.00
Note: HCT-Comorbidity Score is: _____

Signature of person completing form: _____ Date: _____

☐ **FHCRC Patients:**

Patient signed IRB approved consent form. Date: _____

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 **FHCRC** Principal Investigator _____ Date: _____

APPENDIX H

NOTICE OF DEATH

Patient ID: _____ Date of Death: _____

Place of Event: _____

Apparent cause of death (Please be specific. Attach hospital summary or death summary when possible):

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Form completed by: _____ Date: _____

APPENDIX I
SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08
Fred Hutchinson Cancer Research Center
Clinical Research Division
Institutional Review Office

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ ☐ FHCRC/SCCA ☐ Other

Gender: ☐ Male ☐ Female Age: _____

FHCRC Principal Investigator: _____

Phone Number: _____ Mailstop: _____

Date of Report: _____

☐ Initial Report _____ ☐ Follow-Up Report # _____ ☐ Other

Date study staff became aware of event: _____

Date Serious Adverse Event Started:
Date Ended: _____ Or ☐ ~~Ongoing (if ongoing – must submit follow up report)~~

Adverse Event: _____

_____

Describe the Serious Adverse Event including a summary of all relevant clinical information.

(Or attach a MedWatch Form or other SAE reporting form if one has been completed.) Use Page 2, if necessary:

_____

Outcomes Attributed to adverse event: (Check all that apply)

☐ Death / / ☐ Disability
☐ Life-Threatening ☐ Congenital Anomaly
☐ Hospitalization (initial or prolonged) ☐ Required intervention to prevent permanent impairment/damage

Specify Agent(s) and/or Procedure(s) involved in this protocol:

#1 _____ #2 _____

Pharmaceutical product/medical treatment/procedure

☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

☐ Follow-up Report Required

Pharmaceutical product/medical treatment/procedure

☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

☐ Final Report (PI must sign final report)

Report Completed by: _____ Date: _____

The PI has determined that the consent form must be revised: ☐ Yes ☐ No

Does this study involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer)? ☐ yes ☐ no If yes and the activity involves the SCCA outpatient clinic, a copy of this Protocol Modification Form and any supporting documents to be reviewed and approved, will be forwarded to the FHCRC's Institutional Biosafety Committee (IBC) by the Protocol Office (Mailstop: LM-230).

_____ _____

Signature of Principal Investigator Date:

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ Date of Report: _____

Describe the Serious Adverse Event including a summary of all relevant clinical information.

Appendix J

The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI) **9/7/10**

Assign scores appropriately if the patient has any of these comorbidities

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

Instructions: Circle applicable scores and provide actual value or cause of co-morbidity. Fax to FHCRC 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: _____**

2230.00

†One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft.

EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

APPENDIX K

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 degrees 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
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
GASTROINTESTICAL 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., analgesia, nutritional support)	Life-threatening consequences; urgent intervention indicated	Death
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 L
COORDINATING CENTER FUNCTIONS

Outside Center – PI Communication in Hematologic Malignancies

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. The Coordinating Center registers subjects on the study and assigns study IDs
   iii. One copy of the research data is retained by the site. Another data set (identified only by study IDs) is transmitted to the Coordinating Center to create the master data file. All data are kept in locked areas and password protected databases accessible only to study staff
   iv. 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 (FHCRC) within 72 hours if the event occurred during the initial 28 days post NK infusion, otherwise within seven days
   ii. The SAE report is reviewed by the Overall PI. If the SAE meets the FHCRC 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 M
Research Specimens

Pre-Transplant

Recipient:

- 1-2 mL bone marrow aspirate in lavender-top EDTA tube for Immunophenotyping
- 10 mL blood in green-top heparin tube for Immunophenotyping (if lymphopenic, up to an additional 40 ml may be requested)
- 10 mL blood in green-top heparin tube for NK Cytotoxicity Assay
- 10 mL blood in green-top heparin tube for Cell Repository
- 10 mL blood in lavender-top EDTA tube for KIR Genotyping
- 5 mL blood in lavender-top EDTA tube for T Cell Spectratyping and TREC analysis

Donor:

- 10 mL blood in green-top heparinized tube for Immunophenotyping
- 10 mL blood in green-top heparinized tube for Cell repository
- 10 mL blood in green-top heparinized tube for NK cytotoxicity
- 10 mL blood in lavender-top EDTA tube for KIR genotyping
- 5 mL blood in lavender-top EDTA tube for T cell spectratyping and TREC analysis

Day +7:

Recipient:
30-60 minutes PRE-NK infusion

- 10 mL blood in green-top heparin tube for Immunophenotyping

15 minutes from end of NK infusion

- 10 mL blood in green-top heparin tube for Immunophenotyping

Donor (from NK cell final product):

- 0.25 aliquot for immunophenotyping, cryopreservation, and KIR phenotyping

Day +14:

Recipient:

- 10 mL blood in a green top heparin tube for Immunophenotyping

Day +28:

Recipient:

- 10 mL blood in green-top heparin tube for Immunophenotyping
- 10 mL blood in green-top heparin tube for NK cytotoxicity
- 5 mL blood in lavender-top EDTA tube for TREC analysis

2230.00

Day +56:

Recipient:

- 10 mL blood in a green top heparin tube for Immunophenotyping

Day +84:

Recipient:

- 10 mL blood in green-top heparin tube for Immunophenotyping
- 10 mL blood in green-top heparin tube for NK cytotoxicity
- 5 mL blood in lavender-top EDTA tube for T cell spectratyping and TREC analysis

One Year:

Recipient:

- 10 mL blood in green-top heparin tube for Immunophenotyping
- 5 mL blood in lavender-top EDTA tube for T cell spectratyping and TREC analysis

INSTRUCTION FOR RESEARCH LABS

SAMPLES OBTAINED IN OR SENT TO **SEATTLE:**

Distribution of Samples for Processing:

- Heimfeld Lab: David Yaddock, DE 554, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Seattle, WA 98109

Sample Processing at HEIMFELD LAB:

- **Bone marrow aspirate**:
  - **Immunophenotyping**:
    - Arrives: EDTA tube, fresh, room-temperature
    - Action: Perform Immunophenotyping
- **Peripheral blood**:
  - **Immunophenotyping**:
    - Arrives: Heparin tube, fresh, room-temperature.
    - Action: Perform immunophenotyping.
  - **KIR genotyping**:
    - Arrives: EDTA tube, fresh, room-temperature.
    - Action: Sample to be ficolled, PBMC collected, cryopreserved, stored.
    - See section “Every 3-6 months” for shipping instructions
  - **T cell spectratyping and TREC analysis**:
    - Arrives: EDTA tube, fresh, room-temperature.
    - Action: Prepare TREC card (5 spots) and store in ziplock bag at room temperature. Remainder of sample to be ficolled, PBMC collected, cryopreserved, stored.
  - **NK Cytotoxicity Assay:**
    - Arrives: Heparin tube, fresh, room-temperature.

- Action: Ship fresh sample at room temperature via overnight mail to Milwaukee, WI (M-Th only). Email or call lab the day before sample arrival:
- Shipping Address:
  Clinical Research Coordinator – Protocol 2230
  MACC Fund Research Center, Suite 3018
  8701 Watertown Plank Road
  Milwaukee, WI 53226
- Phone/Email:
  - crconc@mcw.edu

- **Cell Repository:**
  - Arrives: Heparin tube, fresh, room-temperature
  - Action: Store sample

- **Every 3-6 Months:**
  - Send batched, TREC cards and cryopreserved PBMC (from ficolled blood samples) to Milwaukee, WI for KIR genotyping, TREC, and T cell spectratyping. Cryopreserved PBMC samples must be sent on dry ice. TREC cards must be doubled bagged (Ziploc-to ensure they stay dry) and placed on TOP of the sealed Styrofoam container (must NOT be packaged in with the dry ice) Email or call lab the day before sample arrival, ship M-Th only.
  - Send with samples: KIR Genotype Invoice that contains Client #3513 and Subject ID#. Put on outside of specimen bag: Completed Sticker for" Diagnostic Laboratories, Blood Center of Wisconsin…"

    - Shipping Address:
      Clinical Research Coordinator – Protocol 2230
      MACC Fund Research Center, Suite 3018
      8701 Watertown Plank Road
      Milwaukee, WI 53226
    - Phone/Email:
      - crconc@mcw.edu

SAMPLES OBTAINED IN OR SENT TO **MILWAUKEE**:

Distribution of samples for processing:

Malarkannan's Lab	Ellis Lab	Verbsky Lab
• Cell Repository • NK Cytotoxicity Assay	• KIR genotyping	• T cell spectratyping and TREC analysis

Sample Processing in HEIMFELD Lab

- **Bone marrow aspirate:**
  - **Immunophenotyping:**
    - Arrives: EDTA tube, fresh, room-temperature
    - Action: Ship fresh sample at room temperature via overnight mail to Heimfeld Lab in Seattle (M-Th only). Email or call lab the day before sample arrival.
    - Shipping Address:
      David Yaddock (Heimfeld Lab Manager)
      DE-554
      Fred Hutchinson Cancer Research Center
      1100 Fairview Ave N
      Seattle, WA 98109
    - Phone/Email:
      - David Yaddock: 206-667-4609; dyadock@fhcrc.org

- **Peripheral blood:**
    - **Immunophenotyping:**
        - Arrives: Heparin tube, fresh, room-temperature.
        - Action: Ship fresh sample at room temperature via overnight mail to Heimfeld Lab in Seattle (M-Th only). Email or call lab the day before sample arrival.
        - Shipping Address:
            David Yaddock (Heimfeld Lab Manager)
            DE-554
            Fred Hutchinson Cancer Research Center
            1100 Fairview Ave N
            Seattle, WA 98109
        - Phone/Email:
            - David Yaddock: 206-667-4609; dyadock@fhcrc.org

Sample Processing in MALARKANNAN Lab:

- **NK Cytotoxicity Assay:**
    - Arrives: Heparin tube, fresh, room-temperature.
    - Action: Perform NK Cytotoxicity Assay
- **Cell Repository:**
    - Arrives: Heparin tube, fresh, room-temperature
    - Action: Store sample

Sample Processing at ELLIS LAB:

- **Peripheral blood:**
    - KIR Genotyping:
        - Arrives: EDTA tube, fresh, room-temperature (if drawn in Milwaukee) or cryopreserved if shipped from Seattle.
        - Action: Perform KIR Genotyping

Sample Processing at VERBSKY/GORSKI LAB:

- **Peripheral blood:**
    - **T cell spectratyping and TREC analysis**:
        - Arrives:
            - Milwaukee sample: EDTA tube, fresh, room-temperature
            - Seattle sample: Cryopreserved +TREC card in ziplock bag
        - Action:
            - Milwaukee sample: (To Verbsky Lab) Prepare TREC card (5 spots) and store in ziplock bag at room temperature in Verbsky Lab for future analysis.
            - Remaining blood sample to be ficolled, PBMC collected, cryopreserved and stored in Verbsky Lab for future T-Cell Spectratyping.
            - Verbsky Lab will deliver cryopreserved blood sample to Gorski Lab for T-Cell Spectratyping.

Appendix N
Intrathecal Therapy Administration

intrathecaltherapy-combined.pdf