AA Phase II Pilot Study of Donor-Derived *Ex-Vivo* Expanded Natural Killer Cell Infusions in Children and Young Adults with High-Risk Acute Myeloid Leukemia Receiving Myeloablative HLA-Haploidentical Hematopoietic Cell Transplant: A Multicenter Pediatric Transplantation and Cellular Therapy Consortium (PTCTC) Study (EXCEL Trial) PTCTC CT2001

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
ADCC	Antibody-dependent cell cytotoxicity
ADL	Activities of daily living
AE	Adverse event
aGVHD	Acute graft versus host disease
ALL	Acute Lymphoblastic Leukemia
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
AP	Alkaline phosphatase
AST	Aspartate transaminase
AUC	Area under the curve
BID	Twice a day
BM	Bone marrow
BMT	Bone marrow transplant
BMT CTN	Bone Marrow Transplant Clinical Trials Network
BSA	Body surface area
Bu	Busulfan
BUN	Blood urea nitrogen
CBC	Complete blood count
CFR	Code of Federal Regulations
cGVHD	Chronic graft versus host disease
cGMP	Current Good Manufacturing Practices
CI	Confidence interval
CIBMTR	Center for International Blood & Marrow Transplant Research
CIK	Cytokine induced killer cells
CMC	Chemistry, Manufacturing, and Control
CML	Chronic myeloid leukemia
CMP	Comprehensive metabolic panel
CMV	Cytomegalovirus
CNI	Calcineurin inhibitors
CNS	Central nervous system
COG	Children’s Oncology Group
CR	Complete remission
CrCl	Creatinine clearance
CRF	Case report form
CSA	Cyclosporine
CSF	Cerebrospinal fluid
Css	Steady state concentration
CTCAE	Common Toxicity Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
Cy	Cyclophosphamide
D_LCO	Diffusing capacity for carbon monoxide
DFS	Disease-free survival
DCI	Donor cellular infusion
DLI	Donor lymphocyte infusion
DLT	Dose-limiting toxicities
DSA	Donor specific antibodies
DSMC	Data Safety and Monitoring Committee
EBV	Epstein-Barr Virus
ECHO	Echocardiogram
EFS	Event free survival

ECOG	Eastern Cooperative Oncology Group
EKG	Electrocardiogram
FAB	French American British
FACT	Foundation for the Accreditation of Hematopoietic Cell Therapy
FDA	U.S. Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FISH	Fluorescence in situ hybridization
FLT3-ITD	FMS-like tyrosine kinase-3/internal tandem duplicate
FVC	Forced vital capacity
FWA	Federal wide Assurance
GCP	Good clinical practice
GFR	Glomerular filtration rate
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
GMP	Good Manufacturing Practice
G-CSF	Granulocyte-colony stimulating factor
GRFS	GVHD/relapse or progression-free survival
GVH	Graft versus host
GVHD	Graft versus host disease
GVL	Graft versus leukemia
HaploHCT	Haploidentical hematopoietic cell transplant
HaploNK	Haploidentical donor natural killer (NK) cells
HBC	Hepatitis B Core total antibodies
HBsAg	Hepatitis B surface antigen
HCT	Hematopoietic stem cell transplantation
HCV	Hepatitis C virus antibodies
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human leukocyte antigen
HSV	Herpes Simplex Virus
HTLV	Human T- cell leukemia virus
HR	Hazard ratio
IB	Investigator Brochure
IBW	Ideal body weight
ICH	International Council for Harmonisation
IDM	Infectious Disease Markers
IFC	Irradiated feeder cells
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISBT	International Society of Blood Transfusion
IT	Intrathecal therapy
LAK	Lymphokine-activated killer cells
LFS	Lung Function Score
LPS	Lansky Performance Status
LVEF	Left ventricular ejection fraction
KIR	Killer cell immunoglobulin-like receptor
KPS	Karnofsky Performance Status
MAC	Myeloablative conditioning regimen
MCB	Master cell bank
MDS	Myelodysplastic syndrome
MFI	Mean fluorescence intensity
MOP	Manual of Procedures
MPA	Mycophenolic acid
MMF	Mycophenolate mofetil
MRD	Minimal residual disease
MUGA	Multigated acquisition scan

NAT	Nucleic acid test
NCH	Nationwide Children’s Hospital
NCI	National Cancer Institute
NIH	National Institutes of Health
NK	Natural killer
NRM	Non-relapse mortality
NS	Normal saline
O_2	Oxygen
OS	Overall survival
PB	Peripheral blood
PBSC	Peripheral blood stem cell
PBMC	Peripheral blood mononuclear cell
PTCTC	Pediatric Transplant and Cellular Therapy Consortium
PCR	Polymerase chain reaction
PI	Principal Investigator
PFS	Progression-free survival
PFT	Pulmonary function test
PK	Pharmacokinetics
PML	Progressive multifocal leukoencephalopathy
PO	By mouth
PRES	Posterior Reversible Encephalopathy Syndrome
PTCy	Post-transplantation cyclophosphamide
RFLP	Restriction fragment length polymorphism
RFS	Relapse-free survival
RIC	Reduced intensity conditioning regimen
RPR	Rapid plasma regain assay
SAE	Serious Adverse Event
SF	Shortening Fraction
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
SOS	Sinusoidal obstruction syndrome
TBI	Total Body Irradiation
TCD	T cell depleted
TID	Three times a day
TNC	Total nucleated cells
TRM	Transplant related mortality
TTP	Thrombotic thrombocytopenic purpura
UCB	Umbilical cord blood
ULN	Upper limit of normal
URD	Unrelated donor
VNTR	Variable number of tandem repeats
VZV	Varicella Zoster Virus
WBC	White blood cell
WCB	Working cell banks
WHO	World Health Organization

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) as adopted by the Food and Drug Administration, USA and United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

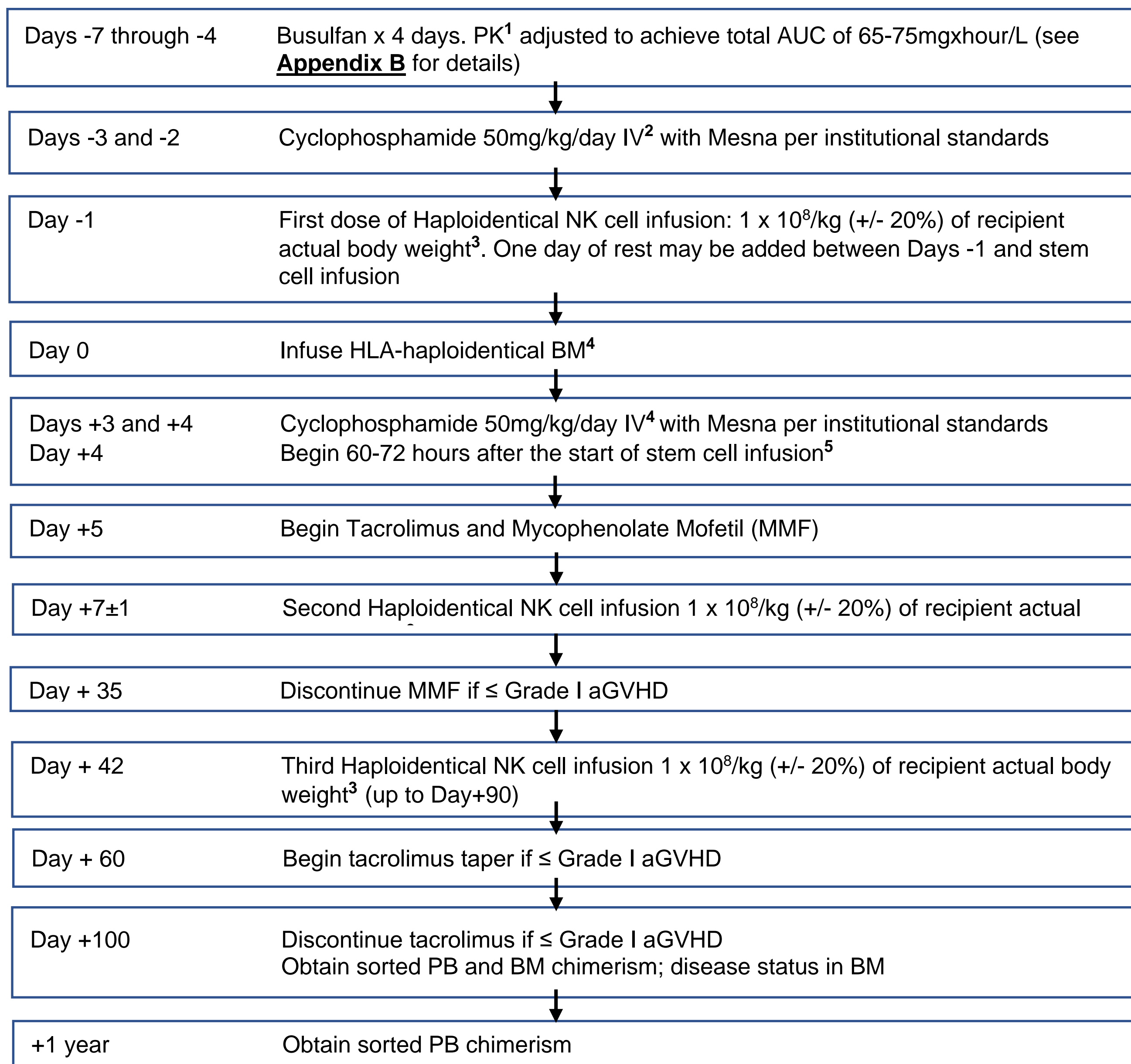
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

PROTOCOL SYNOPSIS

Title:	A Phase II Pilot Study of Donor-Derived *Ex-Vivo* Expanded Natural Killer Cell Infusions in Children and Young Adults with High-Risk Acute Myeloid Leukemia Receiving Myeloablative HLA-Haploidentical Cell Transplant: A Multicenter Pediatric Transplantation and Cellular Therapy Consortium (PTCTC) Study (EXCEL Trial)
Study Description:	This is a Phase II pilot study to determine the efficacy of three fixed dose (1×10^8/kg) infusions of *ex-vivo* expanded human leukocyte antigen (HLA)-haploidentical donor natural killer (NK) cells (haploNK) in children and young adults with high risk acute myeloid leukemia (AML) undergoing HLA-haploidentical hematopoietic cell transplant (haploHCT) with a busulfan and cyclophosphamide-based myeloablative conditioning regimen and post-transplant cyclophosphamide (PTCy) for graft versus host disease (GVHD) prophylaxis. We will also demonstrate the feasibility of performing this trial in a multi-center study. We hypothesize that the infusion of haploNK in this setting will facilitate immune reconstitution and decrease relapse rates and infectious complications without increasing GVHD, resulting in improved survival as compared to recent historical cohorts of haploHCT without NK cell infusion.
Objectives:	Primary Objective: To estimate the 1-year relapse free survival (RFS) in children and young adults with high-risk AML receiving haploNK infusions following a haploHCT.
	Secondary Objectives: • To demonstrate the safety and feasibility of manufacturing and administering donor derived *ex-vivo* expanded haploNK infusions concomitantly with a myeloablative haploHCT in children and adolescents with high-risk AML. • To quantify and characterize NK- and T-cell immune reconstitution and function following HLA-haploidentical HCT and donor-derived *ex-vivo* expanded NK cells at Days +30, +100, +180, and +1 year. • To estimate the incidence of Grade II-IV acute GVHD (aGVHD) (Day +100) and chronic GVHD (cGVHD) (Days +180, +1 year), infections (Days +100, +180), and overall survival (OS) (+1 year and +2 year). • To estimate the incidence of mixed donor chimerism (Days +42, +100, +1 year) • To determine the presence of killer cell immunoglobulin-like receptor (KIR) ligand-ligand mismatch between HLA-haploidentical donor and host and the impact on relapse rate. • To characterize the phenotype and function of the donor-derived *ex-vivo* expanded NK cell product.

Study Population:	• Age ≤ 25 years • An available related HLA-haploidentical donor that is determined to be the most appropriate donor for the patient by the treating physician • One of the following eligible diagnoses: ○ AML in CR1 (defined as <5% blasts in BM by morphology and flow cytometry) having at least one of these high-risk features: ▪ Mutations associated with high-risk disease (**Appendix A**). Other high-risk features not explicitly stated in **Appendix A** can be considered after discussion/approval with the protocol chair/team ▪ MRD-positive at the end of Induction I chemotherapy (defined as flow cytometry ≥ 0.1% blasts) ○ AML in ≥CR2 (defined by <5% blasts in BM by morphology and flow cytometry) • Recovery from prior cycle of chemotherapy as defined by an absolute neutrophil count ≥ 500/mm3 • AML secondary to select germline marrow failure disorders (with exception of Fanconi Anemia) may be eligible but require approval from Protocol Chairs prior to enrollment
Phase:	Phase II/Pilot
Sites/Facilities:	Participating transplant centers of the PTCTC
Enrolling Participants:	Total number of study participants n=30
Study Intervention:	Peripheral blood (PB) ≤ 450 mL and based on donor weight (minimum 10 ml/kg) will be drawn from the HLA-haploidentical donor at least 16 days before the scheduled day of transplant (Day 0). HaploNK cells will be manufactured from the PB of the donor after co-culture with irradiated feeder cells (IFC) as described in **Section 2.4.2**. The recipients will receive three NK cell infusions on Day-1, Day+7 (± 1 day) and Day+42 (up to Day+90) from day of transplant (Day 0).
Accrual Period:	The estimated accrual period is 3 years.
Participant Duration:	Recipients will be followed for 2 years following HCT. Data regarding relapse, survival and GVHD will continue to be collected until the overall study is closed and the clinical dataset is finalized.

TREATMENT SCHEMA



[1] See **Section 2.5.2** for busulfan dosing and **Appendix B** for busulfan AUC conversion table
[2] See **Section 2.5.3** for complete dosing instructions for Cy dosing
[3] See **Section 2.6.2** and **2.6.3** for NK infusion details: Dose 1 should be infused **at least 24h after completion of the last dose of Cy on Day -1**. Dose 2 can be infused on Day +7 ±1 day **at least 24 hours after completion of D+4 PTCY**. Dose 3 can be given any time between Day+42 up to Day+90 from HCT
[4] BM is the preferred stem cell source; PBSCs are allowed only in select cases, see **Section 2.4.1**
[5] See **Section 2.5.5.1** for complete dosing guidelines for PTCy dosing

1.0 BACKGROUND AND RATIONALE

1.1 High-Risk AML is a Challenging Disease to Cure

Acute myeloid leukemia (AML), which constitutes about 15-20% pediatric leukemias, is a heterogeneous disease with respect to morphology, immunophenotyping, underlying germline and somatic genetic abnormalities as well clinical behavior. The incidence of AML is 1.5 per 100,000 individuals per year in infancy, and subsequently decreases to 0.9 per 100,000 in individuals ages 1-4 and 0.4 per 100,00 individuals aged 5-9 years. Rates steadily increase to 16.2 per 100,000 in individuals aged over 65 years[1]. Complete remission (CR) rates as high as 80 to 90% and OS up to 65% are now reported worldwide[2-9] secondary to improvements in supportive care, optimized risk stratification, intensified chemotherapy regimens, and enrollment of children in clinical trials. These interventions have transformed a disease that was once uniformly fatal to one this is now potentially curable. Despite these advances, the cure rate for children with AML lags behind that for children with acute lymphoblastic leukemia (ALL), with the main reasons for treatment failure being relapse and transplant related morality (TRM). The traditional French American British classification (FAB) is based on morphology and immunohistochemistry, whereas the current World Health Organization (WHO) classification of AML considers karyotype, cytogenetic, and molecular aberrations[10,11]. Outcomes are dependent on initial response to treatment, and molecular and cytogenetic aberrations. Treatment consists of a combination of intensive anthracycline and cytarabine-containing chemotherapy regimens and hematopoietic stem cell transplantation (HCT) in selected high-risk cases. In general, 30% of pediatric AML patients will suffer from relapse whereas 5-10% of patients will die due to disease complications or the side effects of treatment[12,13]. Despite improvement in upfront therapy, rates of post-transplant relapse have not improved in AML in over 30 years[14], strongly suggesting that the biology of this disease requires a more robust maintenance platform to maintain remission status (**Figure 1**).

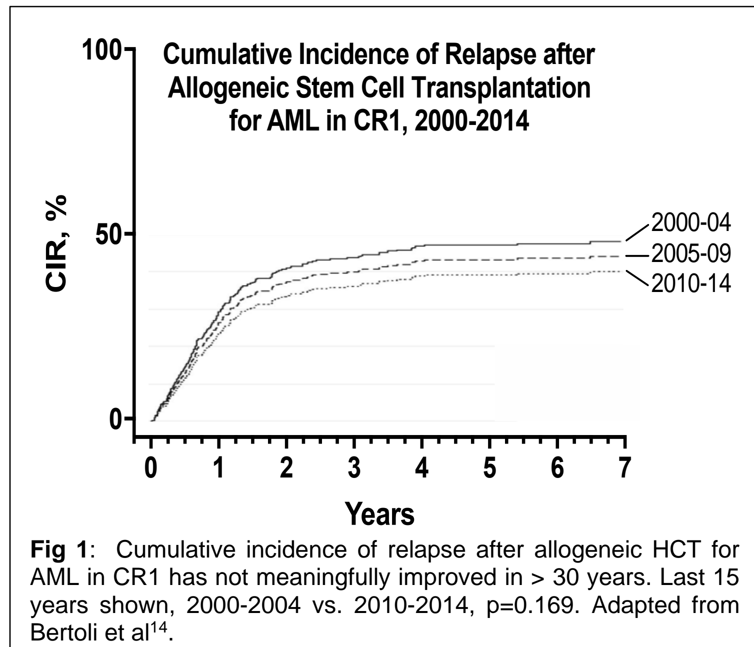


Fig 1: Cumulative incidence of relapse after allogeneic HCT for AML in CR1 has not meaningfully improved in > 30 years. Last 15 years shown, 2000-2004 vs. 2010-2014, p=0.169. Adapted from Bertoli et al[14].

In most children, AML presents as a *de novo* entity but in a minority, it can be due to a secondary malignancy (therapy-related AML). Molecular and cytogenetic markers now enable identification of *de novo* AML with high risk of relapse and historically poor outcomes when treated by chemotherapy alone. These include patients with high allelic ratio of FMS-like tyrosine kinase-3 (FLT3)/internal tandem duplicate (ITD) + status, monosomy 7, or deletion 5q. Additionally, high risk AML has also been defined recently by response to therapy, and minimal residual disease (MRD) status at the end of induction[15]. MRD in AML is typically confirmed via flow cytometry in the United States[16].

1.2 The Role of Minimal Residual Disease Assessment for AML

Traditionally it is preferable to take high-risk AML patients to HCT once they have achieved morphological remission (blasts ≤ 5% in BM). However, with recent sensitive diagnostic techniques (molecular, flow cytometry, cytogenetics), we are now able to detect presence of residual leukemia (1 in 10^5 to 1 in 10^6) in patients who were previously assumed to be in morphological remission. While the prognostic value of MRD in ALL has been extensively studied in pediatric patients, its role in the AML pediatric population continues to be investigated in many recent clinical trials (e.g., Children's Oncology Group (COG) AML1031). Interestingly, the presence of MRD at the end of induction therapy is currently one of the indications to proceed with allogeneic HCT in the above study.

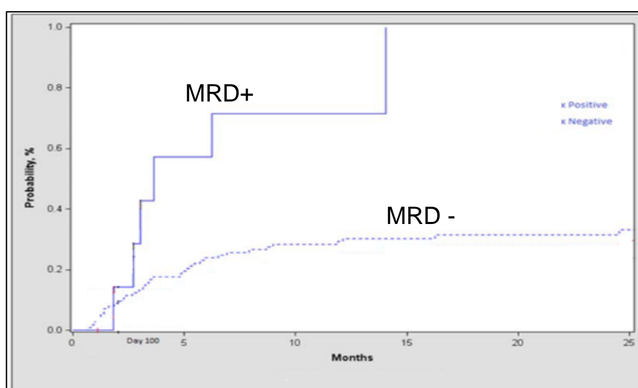


Fig. 2: Probability of relapse in children and adolescents with AML undergoing HCT, stratified by pre-HCT MRD+ or MRD-.

The challenge remains that there are no recommendations regarding how to proceed if patients are MRD+ just prior to HCT. Few studies have examined the role of pre-transplant MRD and its impact on outcomes after HCT in pediatric AML[17,18]. Presence of MRD may lead to further intensification of therapy prior to HCT and may also provide the rationale for other maintenance therapies after HCT including donor lymphocyte infusion (DLI) and NK cell therapy. MRD by flow cytometry measurements was introduced in the AML0531 but was not used as deciding factor to determine if the patient should proceed with transplant or not. In the recently closed AML1031 COG trial, patients with standard risk cytogenetics with positive MRD at the end of Induction I chemotherapy were also identified as high risk and were allocated to receive HCT as a post-remission consolidative therapy. In a multicenter study of 124 pediatric AML patients, MRD by multi-dimension flow cytometry pre HCT and at Day +42 and Day+100 was found to be predicative of clinically significant outcomes. Most notably, patients with pre HCT MRD positivity had a 2-year relapse rate of 100% vs 32% in those who were negative (**Figure 2**). Similarly, the 2-year disease-free survival (DFS) and OS was 0% and 29% for positive MRD pre HCT and 54% and 62% of negative MRD[18].

1.3 Hematopoietic Cell Transplantation for AML

1.3.1 HCT in High-Risk *de novo* and Relapsed AML and Therapy-Related AML

In high-risk AML, HCT often abrogates the poor outcomes seen with chemotherapy alone. In these situations,[19], DFS is 69%, which is comparable with patients with standard risk AML treated with chemotherapy alone. HCT continues to be the only remission-intensification treatment of choice in patients with relapsed and therapy-related AML, with reported OS rates of 30-61%[20-25] and 22-32%[26-30] , respectively. The benefit of HCT is in part due to the delivery of a leukemia-free graft and the graft versus leukemia (GVL) effect mediated by the emergence of donor natural killer (NK) cells in the post-HCT setting[31-34]. Donor NK cells are the first cells to recover post HCT. The long-term relapse-free survival (RFS) that is afforded by HCT for myeloid malignancies is associated with immunologic parameters that predict NK cell function and alloreactivity. The impact of NK cells on survival has been associated with increased numbers of NK cells in the stem cell graft, rapid recovery of NK cells in the early post-transplant period, alloreactivity of NK cells in the GVH direction predicted by killer cell immunoglobulin-like receptor (KIR)-ligand mismatch, more refined measures of mismatch that include KIR genotyping, activating KIR gene content, assigned KIR genotypes, and presence or absence of specific KIR genes[32,34-40]. These areas of mismatch are further enhanced when using HLA-haploidentical donors.

In a recent retrospective study in children by Center for International Blood & Marrow Transplant Research (CIBMTR), the 5-year probability of OS following either reduced-intensity conditioning (RIC) or myeloablative conditioning (MAC) regimens was 45% and 48% respectively. Post-transplant relapse continues to be a major cause of death in 39% of patients[41]. The frequency of treatment- related deaths (5-10%) both for newly diagnosed as well for relapsed patients, as well as the development of chemotherapy-related late-effects, precludes further intensification of pre-HCT chemotherapy to prevent relapse.

Therefore, there is an urgent need for new therapeutic maintenance approaches which cause less severe side effects in this setting.

1.3.2 HLA-Haploidentical HCT for AML

A major improvement in the field of hematopoietic cell transplantation (HCT) is the use of HLA-haploidentical donors with PTCy for graft versus host disease (GVHD) prophylaxis. The use of haploidentical donors increases the availability of transplantation for nearly all patients, and the application of PTCy significantly improves infectious complications and GVHD rates, resulting in survival rates that approximate those of matched unrelated donor transplants[42]. Non-relapse mortality (NRM) of haploHCT has dropped to 5-10%, but relapse remains the primary cause of treatment failure with 1-year relapse incidence of 25-45%[43-47]. Thus, measures aimed at reducing relapse that do not increase NRM will significantly improve the efficacy of this regimen.

The Pediatric Transplantation and Cellular Therapy Consortium (PTCTC) recently initiated a multi-site Phase II study of PTCy in haploHCT for both lymphoid and myeloid hematological malignancies based on the Johns Hopkins experience with Cy-total body irradiation (TBI) and Bu-Cy as conditioning regimens (PTCTC study ONC1402), respectively. This myeloid group will serve as a baseline cohort with which to compare the safety, feasibility, and efficacy of adding donor NK cell infusions to this same haploHCT platform in pediatric patients. This PTCTC trial also collected research samples evaluating NK cell reconstitution, and so it will be possible to use this data to compare NK cell biological correlatives in the current trial.

In a study published by Russo et al[48], high levels of serum interleukin (IL)-15 were noted early post-HCT in patients who underwent haploHCT followed by PTCy (**Figure 3**). This is of interest as IL-15 is an important cytokine for NK cell survival and activation[49]. Thus, this burst of innate IL-15 production after PTCy can be particularly efficacious as a strategy to further expand and activate donor NK cells. However, although NK cell numbers increase early post-HCT and are likely all of donor-origin, this study reported another important observation that the NK cell phenotype remains mostly immature until at least 6-12 months after HCT. **Thus, adoptive immunotherapy strategies that can employ multiple infusions of mature NK cells after HCT in the PTCy setting might mediate necessary GVL reactions until full immune reconstitution can occur.**

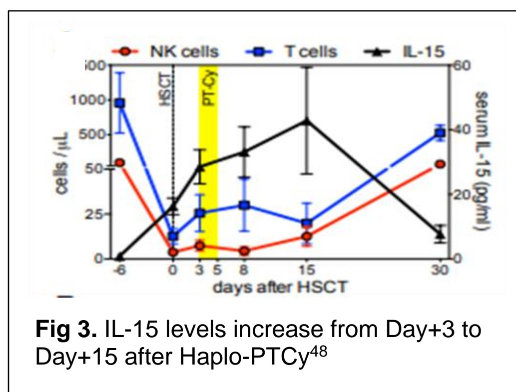


Fig 3. IL-15 levels increase from Day+3 to Day+15 after Haplo-PTCy[48]

1.4 Natural Killer Cells

1.4.1 Biology of NK Cells

NK cells represent 5-15% of PB lymphocytes[50], but comprise 85% of the large granular lymphocyte population[51]. Having both cytotoxic and regulatory activity, NK cells recognize virus-infected or malignant cells that express danger signals (e.g., stress ligands, viral proteins, antibodies). Their participation in recognizing cancer through antibody-dependent cell cytotoxicity (ADCC)[52] is a major mechanism of the therapeutic benefit of anti-cancer antibodies. To control reactivity against self, NK cells have inhibitory receptors for self HLA, and therefore have heightened sensitivity to cells that lack class I HLA[53].

NK cells were first described in the 1970s based on their functional ability to kill tumor cells without prior sensitization[51]. In their resting state, they mainly circulate in the blood and hematopoietic tissues, but NK cells can also be isolated from tissues such as the liver, peritoneal cavity, and placenta. Upon activation, they extravasate into surrounding tissue to affect killing of pathogen-infected or neoplastic cells. Their recognition of target cells is not restricted by presentation of antigens through HLA, and is not antigen specific, differentiating them from T cells. Until the 1980s, they were identified as lymphocytes lacking the receptors of B cells or T cells, giving rise to the name "null cells." Therapeutic use was largely in the context of heterogeneous activated lymphocyte populations, as they are a key active component of lymphokine-activated killer cells (LAK)[54,55], and cytokine-induced killer cells (CIK) , both of which possess NK cell-like phenotype and function[56]. About a decade after their initial discovery, these cells were identified as $CD3^{neg}$

lymphocytes (i.e., not T cells) that express CD56. This remains the most common definition for human NK cells, although NKp46, an activating receptor broadly expressed on mature NK cells, may define NK cells more uniformly across species[57].

NK cells play a central role in tumor immunity through many of the same mechanisms as viral immunity. NK cells exhibit direct cytotoxicity against malignant cells that is critical to the priming of the adaptive immune response by releasing tumor antigens that can be processed and presented to T cells. In addition, release of pro-inflammatory cytokines such as IFNγ recruits T cells to the tumor site, promotes T-cell and dendritic cell activation, and enhances humoral responses by promoting B-cell maturation[58].

1.4.2 NK Cell Expansion Methods

The inability to obtain large numbers of pure NK cells has limited investigators in moving forward in testing the potential efficacy of NK cell therapy. Thus, the major unmet need limiting NK cell adoptive immunotherapy is a method for obtaining and delivering large enough numbers of cells to be effective, with current approaches limited by the high cost, donor inconvenience, and low number available from donor apheresis (~10^7 cells/kg), and low proliferation (< 1,000-fold) under current expansion methods.

Previously, NK cell expansion with IL-15 was limited by short-telomere-induced senescence. We developed a novel *ex-vivo* expansion method for NK cells that resolves this problem[59,60]. This method uses a feeder cell genetically engineered to express membrane bound IL-21 (mbIL21, which signals primarily through STAT3), and 4-1BB ligand (which provides NK cell co-stimulation). The combination of signals promotes sustained proliferation of mature NK cells without senescence by increasing the telomere length in the expanded cells. The method enables large-scale expansion of NK cells from a small volume of PB, sufficient to deliver multiple infusions of NK cells at high cell doses[59]. These expanded NK cells display a highly-activated phenotype (CD16or56^{pos}/CD3^{neg}) and demonstrate high cytotoxicity against many cancer cell lines *in vitro* [61]. NK cell expansion *ex-vivo* achieves a mean of >3,000-fold expansion in 14 days, sufficient to generate enough NK cells from 1 unit of PB to deliver at least 6 infusions of 10^8 cells/kg[62,63]. This expansion is many-fold greater than the highest expansion published to date[64,65], enabling the infusion of many more NK cells than yet reported[66,67]. In preparation for this clinical trial and others, we manufactured the feeder cell CSTX002 in the NCH/OSU GMP facilities as a clinical grade master cell bank (MCB). This cell bank and/or its derivative working cell banks (WCB) will be used for the propagation of NK cells in this multi-institution trial for AML.

1.4.3 Adoptive Transfer of NK Cells

With broad anti-cancer potential, adoptive immunotherapy with NK cells has emerged as a promising anti-cancer treatment. NK cells have therapeutic potential for a wide variety of human malignancies, including sarcomas[68,69], myeloma[70], carcinomas[35,71-73], lymphomas[74], and leukemias[35,75,76]. Adoptive transfer of NK cells has been shown to be safe in adult patients with hematological malignancies[31,35] and high numbers of NK cells in allogeneic hematopoietic cell grafts are associated with improved outcomes[77] supporting the use of NK cells as a therapeutic strategy. Two clinical observations provided initial rationale for NK cells demonstrating an anti-tumor effect. The first is based on the observation that recovery of NK cells after HLA-haploHCT improves tumor-free survival for patients with AML[39]. The second was that adoptive transfer of IL-2 activated haploidentical NK cells after lympho-depleting chemotherapy can lead to a complete response in patients with advanced AML[35]. To date, most clinical studies exploiting NK cells for adoptive immunotherapy have been performed with NK cells isolated from leukapheresis products using immunomagnetic cell selection[78,79] and overnight IL-2 activation[80]. This process enabled NK cell adoptive immunotherapy, but is expensive, invasive, and remains limited in cell dose to a single infusion of typically less than 2 x 10^7 NK cells/kg[80]. Using this approach, Miller et al[35] demonstrated that infusion of haploNK cells after chemotherapy could induce remission in poor-prognosis AML patients, and remission was associated with KIR mismatch. In a similar study, Rubnitz et al[76] reported the safety of KIR-mismatched NK cell infusion as post-remission consolidation therapy for children with AML[76], with no relapses reported in the 10 patients treated. A similar approach has been used for adoptive transfer of NK cells in patients with refractory lymphoma[74] and multiple myeloma[67]. GVHD was not reported in any of these studies.

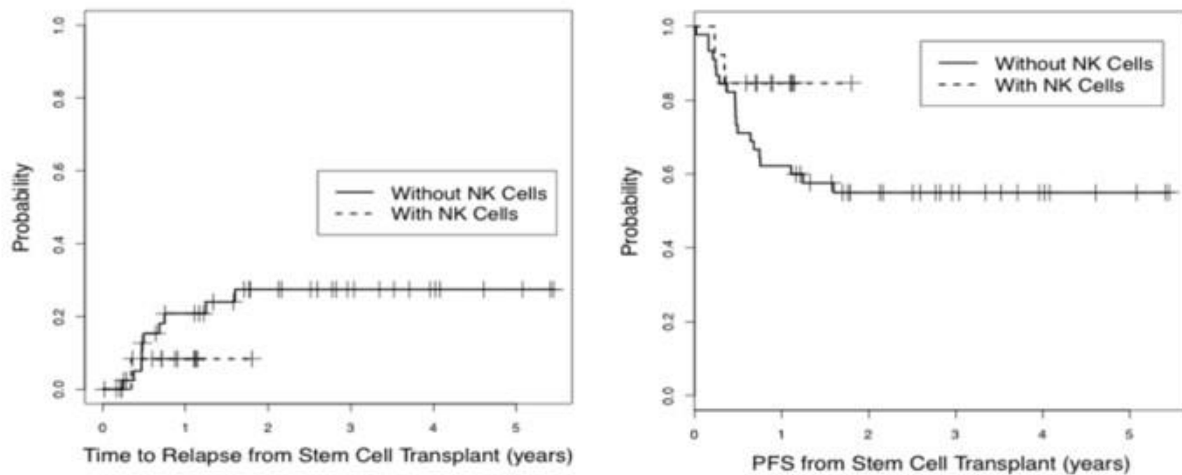


Fig 4: Relapse (left) and PFS (right) of adult patients with myeloid malignancies that received 9dotted line) or did not receive (solid line) donor NK cell infusion after haploHCT with PTCy. All 13 patients from the Phase I study are shown. All patients were treated at MD Anderson with the same fludarabine-melphalan conditioning regimen.

NK cells are an important part of the GVL effect in haploHCT. As previously described, we and others have shown that NK cell reconstitution in the PTCy setting is severely compromised[63,81], leading us to hypothesize that infusion of mature NK cells in the peri-transplant period could compensate for the poor NK cell number and function observed early after haplo-HCT. To test this hypothesis, we conducted a Phase I dose-escalation study in adults with high-risk myeloid malignancies infusing *ex-vivo* expanded donor-derived NK cells on Days-2, +7 and ≥ 28 after RIC haploHCT[63]. Dose escalation was 10^5 to 10^8 NK cells/kg. Strikingly, compared to the historical MD Anderson controls of identically treated patients who did not receive NK cells, in our Phase I study we observed improved progression-free survival (PFS) (85% vs. 55%) and a reduction in leukemia relapse (7% vs. 35%, (**Figure 4**). However, neither of these measures met significance in this small, Phase I pilot study. There was also a trend toward less BK virus cystitis (0% vs. 25%,

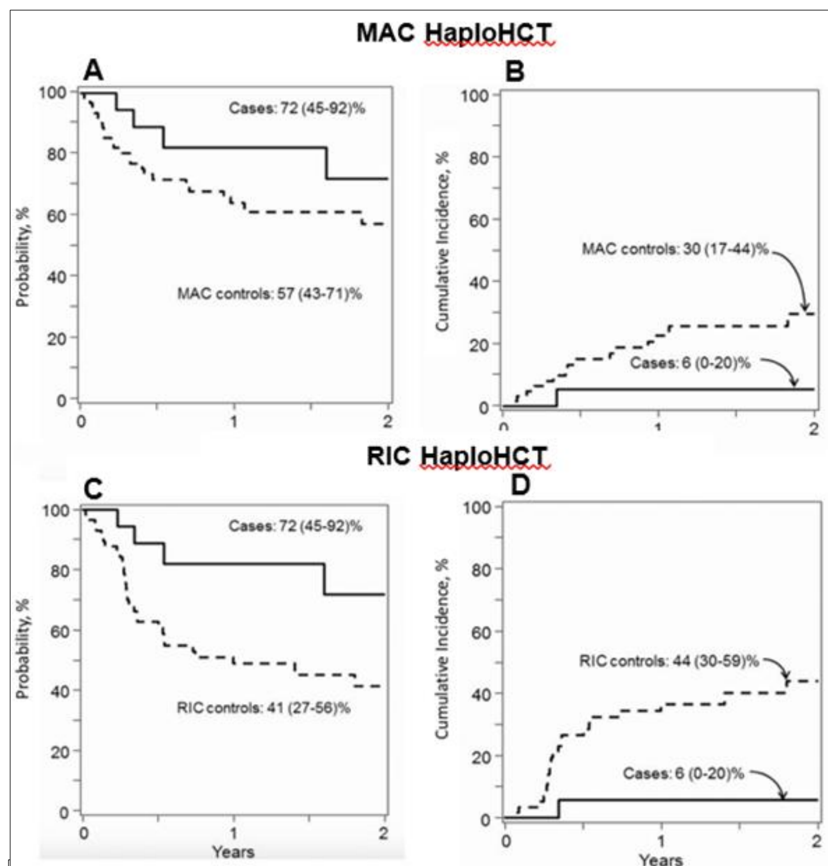


Fig. 5: RFS (left A & C) and relapse incidence (right B & D) of adult AML/MDS patients receiving haploHCT with NK cells on Phase I and Phase II studies (solid line, n = 18) compared to CIBMTR registry case-matched controls (4:1 where possible, dotted line) of haploHCT following myeloablative (upper, n = 63) or reduced-intensity (lower, n = 57) conditioning regimens.

p=0.11) and cytomegalovirus (CMV) reactivation (30.8% vs. 70.4%, p=0.01), both of which are major contributors to morbidity and mortality post HCT.

This trial has continued to accrue in a Phase II expansion, and interim comparison of AML/MDS patients was performed with case-matched cohorts of patients receiving haploHCT with PTCy, identified through the CIBMTR patient database, selected from those receiving either MAC or RIC regimens, respectively. 1-year RFS in the RIC arm was similar to the MD Anderson comparison cohort at 41%, and in the MAC arm was 57% [82]. Compared to these CIBMTR matched cohorts, the RFS and relapse rates were considerably improved in those who received NK infusions on study (**Figure 5**).

Based on the findings of this study we propose testing the safety and feasibility of infusing haploNK cells at a fixed dose of 10^8/kg/dose of NK cells recipient actual body weight on Days -1, +7 and +42 post HCT in pediatric patients with high-risk AML undergoing myeloablative haploHCT with PTCy.

1.4.4 Rationale for NK Cell Dose and Timing

Three Phase I clinical trials have been completed using NK cells expanded with feeder cells expressing mbIL21 and 4-1BB ligand in patients with multiple myeloma undergoing autologous HCT (NCT01729091[83]), with AML/MDS receiving haplo HCT (NCT01904136[63]), and myeloid malignancies under-going matched allogeneic HCT (NCT01823198). A Phase II extension of the haploHCT trial and two additional Phase I studies in relapsed/refractory AML (NCT01787474) and pediatric brain tumors (NCT02271711) are in progress. This approach has also been developed in Brazil for an ongoing clinical trial that parallels the US Phase I study for relapsed/refractory AML (NCT02809092).

In NCT01729091, 29 patients with multiple myeloma undergoing autologous HCT with melphalan received lenalidomide and infusions of NK cells expanded from umbilical cord blood (UCB) in doses ranging from 5 x 10^6 to 10^8/kg. Twelve patients treated during the Phase I dose escalation were reported. One patient had graft failure attributable to poor graft quality. There were no toxicities with the NK cell infusions. Toxicities experienced during the Phase II portion of the study have not yet been reported.

In the ongoing Phase I/II study NCT01904136, 25 patients have been treated to date with myeloid malignancies (AML, MDS, CML) receiving a haploidentical donor HCT have been treated in an ongoing protocol that delivers three infusions of NK cells (on Days-2, +7, and +28) expanded from PB of the donor in doses ranging from 10^4 to 10^8/kg[63,82]. Thirteen patients treated during the Phase I dose escalation were reported[63]. All but two patients received all three planned infusions, and these were held for concurrent GVHD. No patients developed more than Grade II GVHD, which was less than the historic rate for this regimen without NK cells. One graft failure, associated with infection, was observed in the patient treated at 10^4/kg. No dose-limiting toxicities (DLT) have been observed in the Phase I or Phase II components, and non-DLT adverse events have been minimal, such as transient fever, rash, and tachycardia, but formal summary reports of toxicities are not yet available (personal communication, S. Ciurea).

Twenty-two patients with myeloid leukemias undergoing matched allogeneic HCT received infusions of NK cells expanded from either UCB (n=10) or PB of related HLA-matched (N=9) or haploidentical (N=3) donors in doses ranging from 10^6 to 10^8/kg (NCT01823198). No DLTs were observed, and non-DLT adverse events were minimal, such as transient fever, rash, and tachycardia, but formal summary reports of toxicities are not yet available (personal communication, R. Champlin).

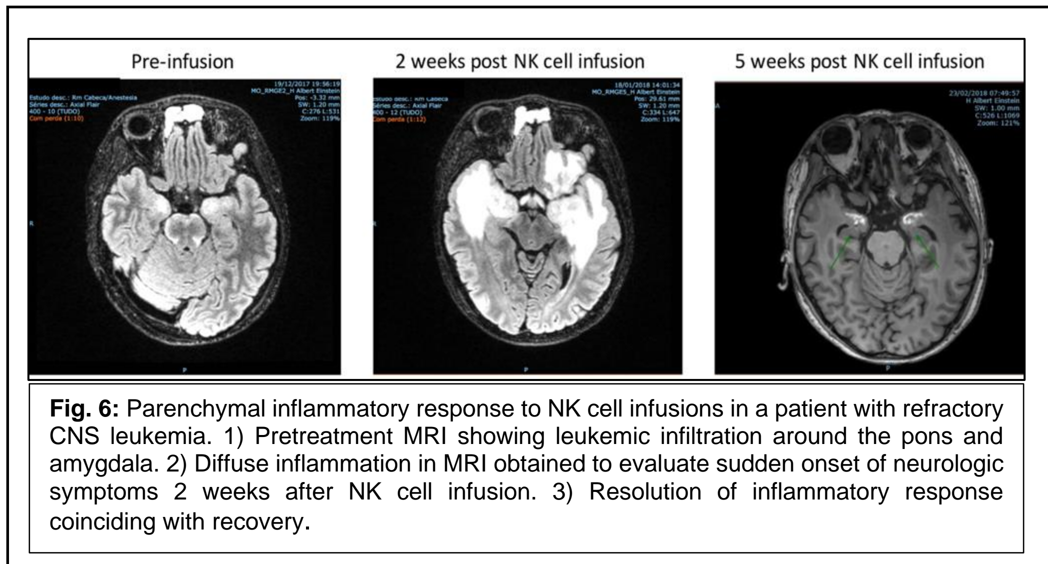


Fig. 6: Parenchymal inflammatory response to NK cell infusions in a patient with refractory CNS leukemia. 1) Pretreatment MRI showing leukemic infiltration around the pons and amygdala. 2) Diffuse inflammation in MRI obtained to evaluate sudden onset of neurologic symptoms 2 weeks after NK cell infusion. 3) Resolution of inflammatory response coinciding with recovery.

In the ongoing Phase I studies NCT01787474 in USA and NCT02809092 in Brazil[84]), 17 patients having relapsed and/or refractory AML received reinduction chemotherapy (fludarabine/ cytarabine +/- G-CSF) followed by six infusions of expanded haploNK cells (thrice weekly for two weeks) in doses ranging from 10^6 to 10^7/kg. One patient with resolving hepatitis and ascites at enrollment died from exacerbation of the hepatitis and ascites. This was felt to be related to the reinduction chemotherapy; but, was attributed as possibly related to the NK cells (personal communication, S. Ciurea). A patient with active refractory CNS leukemia at the time of enrollment experienced headaches, mental status changes, and ataxia 1 weeks after the last NK cell infusion. An MRI showed massive inflammation at the location of known CNS disease. He received high dose steroids and slowly recovered without residual deficits and remained in remission for 9 months. The toxicity was attributed to NK cells and associated with their therapeutic benefit [85] (**Figure 6**).

1.4.4.1 Dose of NK Cell Infusions

In our Phase I dose-escalation study[63] in adults with high-risk myeloid malignancies, 11 of the 13 intended recipients received all 3 planned haploNK cell infusions ranging from 1 x 10^5/kg to 1 x 10^8/kg of recipient weight. No infusion-related reactions or dose limiting toxicities were observed. In addition, there was no increased risk of severe aGVHD or cGVHD compared to historical controls. Only 1 of the 13 patients relapsed at last follow up and this patient was treated at the lowest investigational dose (1 x 10^5 NK cells/kg per dose). That adult study has continued to accrue patients in the companion Phase II trial[82] and an additional 14 patients have been treated at the 1 x 10^8/kg dose level with no infusion related toxicities or GVHD (PI communication). Therefore, we in our current study propose testing the safety and feasibility of infusing haploNK cells at a fixed dose of 1 x 10^8/kg (+/- 20%) of recipient actual body weight.

1.4.4.2 Timing of NK Cell Infusions

We propose delivering expanded donor NK cells at three time points:

- Day-1 prior to the stem cell infusion
- Day+7 after the stem cell infusion
- Day+42 after the stem cell infusion

Several lines of evidence have been combined to suggest this timing of NK cell infusions.

Day-1: NK cell content in the stem cell graft is associated with improved outcomes[31,77,86,87]. Murine models suggest that infusion of alloreactive NK cells may promote stem cell engraftment [88-90] and may decrease the incidence of GVHD through a mechanism of deleting host antigen presenting cells[39] or directly inhibiting host T cells[91], thereby blocking early cross-priming of T cells to alloantigens. Lastly, AML blasts have heightened sensitivity to NK cell lysis from metabolic and genotoxic stress of chemotherapy[92,93].These all support the infusion of large numbers of NK cells at the same time as, or shortly before, the stem cell

infusion. We chose to deliver the first dose of NK cells at Day-1 in order to be at least 24 hrs after the last dose of chemotherapy and to not interfere with the stem cell infusion.

Day+7: As mentioned above, early NK reconstitution after HCT is associated with DFS. However, while PTCy greatly reduces the incidence of GVHD it also has a major adverse impact on NK cell reconstitution[48]. In addition, the *in vivo* expansion of NK cells is associated with high serum concentrations of IL-15[35], and high IL-15 levels are observed in patients at Day+7 who have received PTCy[48]. Thus, the Day+7 environment is optimal for NK cell infusions, and we demonstrated that reconstitution was improved in this setting by infusing NK cells shortly after Cy[63].

Day+42: NK cell reconstitution around Day+28 is associated with improved RFS. Although NK cell number and function are greatly improved by the NK cell infusion at Day+7[63], most pre-clinical and clinical observations suggest that adoptively-transferred NK cells last only a few weeks. Furthermore, immune reconstitution has been shown to be affected in the early PTCy environment[48] suggesting an additional boost of mature cells could be beneficial. Finally, MMF, while a mainstay in this haplo-HCT regimen, is likely to inhibit NK cell function[94]. As MMF will be discontinued on Day+35 on this protocol, we will infuse the third dose of NK cells one week later on Day+42. This additional infusion of NK cells at Day+42 will further enhance NK cell activity.

1.4.5 Study Summary

To summarize, relapse continues to be the major cause of treatment failure in children with high-risk AML post allogeneic HCT[14]. NK cells are the first immune cells to recover post-HCT and mediate a powerful GVL response. HaploHCT with PTCy for GVHD prophylaxis has become a well-established HCT approach for patients who lack HLA-matched donors. However, NK cell recovery is severely impaired in this setting and relapse rates remain high[48]. To date, NK cell adoptive immunotherapy has been limited by low numbers of cells available from donors. To solve this, we have engineered a novel NK cell expansion method utilizing genetically engineered feeder cells, sufficient to deliver multiple NK cell infusions at 1 x 10^8 cells/kg/dose. In adults, restoring NK cell function by infusing these expanded and activated donor NK cells in patients with poor-prognosis AML after haplo-HCT with PTCy was associated with decreased risk of relapse[63]. However, this sequential approach of three expanded NK cell infusions has never been studied in children undergoing haplo-HCT. In our proposed multicenter Phase II pilot trial, we will study the safety, feasibility, and efficacy of expanded donor NK cell infusions (on Day-1, Day+7 and Day+28) post haplo-HCT with PTCy in children with high-risk AML. The results of this trial will provide proof-of-principle for NK cell adoptive immunotherapy in improving post-HCT survival of children and young adults with high-risk AML.

2.0 STUDY DESIGN

2.1 Objectives

2.1.1 Primary Objective

To estimate the 1-year relapse free survival (RFS) in children and young adults with high-risk AML receiving donor-derived NK cell infusions following a haploHCT.

2.1.2 Secondary Objectives

1. To demonstrate the safety and feasibility of manufacturing and administering donor derived *ex-vivo* expanded haploNK infusions concomitantly with a myeloablative haploHCT in children and adolescents with high-risk AML.
2. To quantitate and characterize NK- and T-cell immune reconstitution and function following HLA-haploHCT and donor-derived *ex-vivo* expanded NK cells at Days+30, +100, and +180, and +1 year.
3. To estimate the incidence of Grade II- IV aGVHD (Day +100) and cGVHD (Day+180, +1 year), infections (Days +100, +180), and OS (+1 year and +2 year).
4. To estimate the incidence of mixed donor chimerism (Day +42, +100, +1 year).
5. To determine the presence of KIR ligand-ligand mismatch between HLA-haploidentical donor and host and the impact on relapse rate.
6. To characterize the phenotype and function of the donor-derived *ex-vivo* expanded NK cell product.

2.2 Rationale for Study Design

Building on the experience of PTCTC study ONC1402, we hypothesize that adding three infusions of *ex-vivo* expanded HLA-haploidentical donor NK cells to a myeloablative conditioning regimen consisting of busulfan and Cy will decrease relapse rates and improve survival in pediatric patients transplanted for high-risk AML. We further hypothesize that immune reconstitution will be improved without causing increased GVHD.

2.3 Eligibility

2.3.1 Recipient Inclusion Criteria

1. Age ≤ 25 years at time of enrollment
2. High-risk AML, as defined by one of the following:
    a. AML in CR1 (defined as <5% blasts in BM by morphology and flow cytometry) having at least one of these high-risk features:
        i. Mutations associated with high-risk disease (**Appendix A**). Other high-risk features not explicitly stated in **Appendix A** can be considered after discussion/approval with the protocol chair/team
        ii. MRD-positive at the end of Induction I chemotherapy (defined as flow cytometry ≥ 0.1% blasts)
    b. AML in ≥CR2 (defined by <5% blasts in BM by morphology and flow cytometry)
3. Recovery from prior cycle of chemotherapy as defined by an absolute neutrophil count ≥ 500/mm^3
4. AML secondary to select germline marrow failure disorders (with exception of Fanconi Anemia) may be eligible but require approval from Protocol Chairs prior to enrollment.
5. Performance status ≥70% (Lansky for <16 years; Karnofsky for ≥16 years)
6. Adequate major organ system function as demonstrated by:
    a. Renal: Creatinine clearance (CrCl) ≥60 mL/min/1.73m^2 by Cockcroft-Gault formula, Schwartz formula, or nuclear GFR study (**Table 3**)
    b. Hepatic: Total bilirubin <2 mg/dL (unless due to Gilbert syndrome) and ALT and AST < 5x ULN
    c. Cardiac: LVEF at rest ≥50% or SF ≥27% (by MUGA or ECHO)
    d. Pulmonary: DLCO, FEV_1, and FVC ≥ 50% of predicted corrected for hemoglobin. For patients <7 years of age or those unable to perform PFTs: O_2 Sat >92% on room air by pulse oximetry and on no supplemental O_2 at rest
7. The patient, patient's parent, guardian, or legal representative can provide written informed consent

2.3.2 Recipient Exclusion Criteria

1. Active extramedullary disease
2. Unresolved/ongoing and serious viral, bacterial, or fungal infection despite appropriate treatment
3. Positive pregnancy test in a female of child-bearing potential (FCBP)
4. Inability to comply with medical therapy or follow-up
5. Prior allogeneic transplant
6. Patients with Fanconi Anemia and Down syndrome

2.3.3 Donor Inclusion Criteria

1. HLA haploidentical (≥ 4/8 and ≤ 7/8 allele match at the -A, -B, -C, -DRB1 loci) by high-resolution HLA typing and related to the patient
2. Weight ≥ 50kg
3. Age ≥ 17 years
4. Willing and able to undergo:
    a. PB collection for NK cell production approximately 2-3 weeks before the recipient's admission for transplant
    b. BM harvest (PBSC allowed in rare cases per **Section 2.4.1**)
5. Meets current 21 CFR 1271 criteria for donor eligibility of therapeutic cells and hematopoietic stem cells

Refer to **Section 2.3.5** for recommendations related to HLA-haploidentical donor prioritization.

2.3.4 Donor Exclusion Criteria

1. Per treating physician, determined unable to discontinue medication likely to interfere with adequate NK cell expansion (e.g., high dose steroids or immunosuppression) given at the time of PB collection for NK cell production

2.3.5 Donor Prioritization Schema

Please note: **An unrelated donor (URD) search is not required prior to enrollment on this protocol. Furthermore, having an available HLA-matched related or URD does not make a subject ineligible for this protocol.**

Local institutional criteria are to be used in choosing the most appropriate HLA-haploidentical donor. In absence of local criteria, the following suggestions, in order of importance, are recommended for HLA-haploidentical donor selection:

1. Absent or low patient donor-specific antibodies (DSA)
    a. Specifically, complement dependent cytotoxicity and flow cytometric crossmatch assays must be negative, and the mean (or median) fluorescence intensity (MFI) of any anti-donor HLA antibody by solid phase immunoassay should be <2000.
        i. If a screening assay against pooled HLA antigens is used, positive results must be followed with specificity testing using a single antigen assay. The MFI must be <2000 unless the laboratory has validated higher threshold values for reactivity for HLA antigens (such as HLA-C, -DQ, and -DP), that may be enhanced in concentration on the single antigen assays.
        ii. Consult with Protocol Chairs for the clinical significance of any anti-donor HLA antibody.
        iii. If centers are unable to perform this type of testing, please contact the Protocol Chairs to make arrangements for testing.
        iv. ***In patients with limited donor options, and high level of DSA (i.e., MFI by solid phase immunoassay of >2000), any antibody depletion protocol/strategies must be discussed with the Protocol Chairs.***
2. ABO compatibility (in order of priority):
    a. Compatible or minor ABO incompatibility
    b. Major ABO incompatibility
3. CMV serostatus:
    a. For a CMV seronegative recipient: the priority is to use a CMV seronegative donor when feasible
    b. For a CMV seropositive recipient: the priority is to use a CMV seropositive donor when feasible
4. Age: ≥17 years of age
5. Donor body weight sufficient for adequate marrow collection per patient size, though donor considered non-obese (per BSA measurement)
6. Male donors preferred over female nulliparous donors over female parous/multiparous donors
7. If performed at site: KIR status by mismatch, KIR-B, or KIR content criteria according to institutional guidelines.

2.4 Treatment Plan

2.4.1 Donor Process

Screening and testing for suitability of haploidentical donors will conform to current 21 CFR 1271.
Donor screening must occur (including infectious disease testing) within 7 days of PB collection for NK cell production and within 30 days of stem cell collection for transplant.

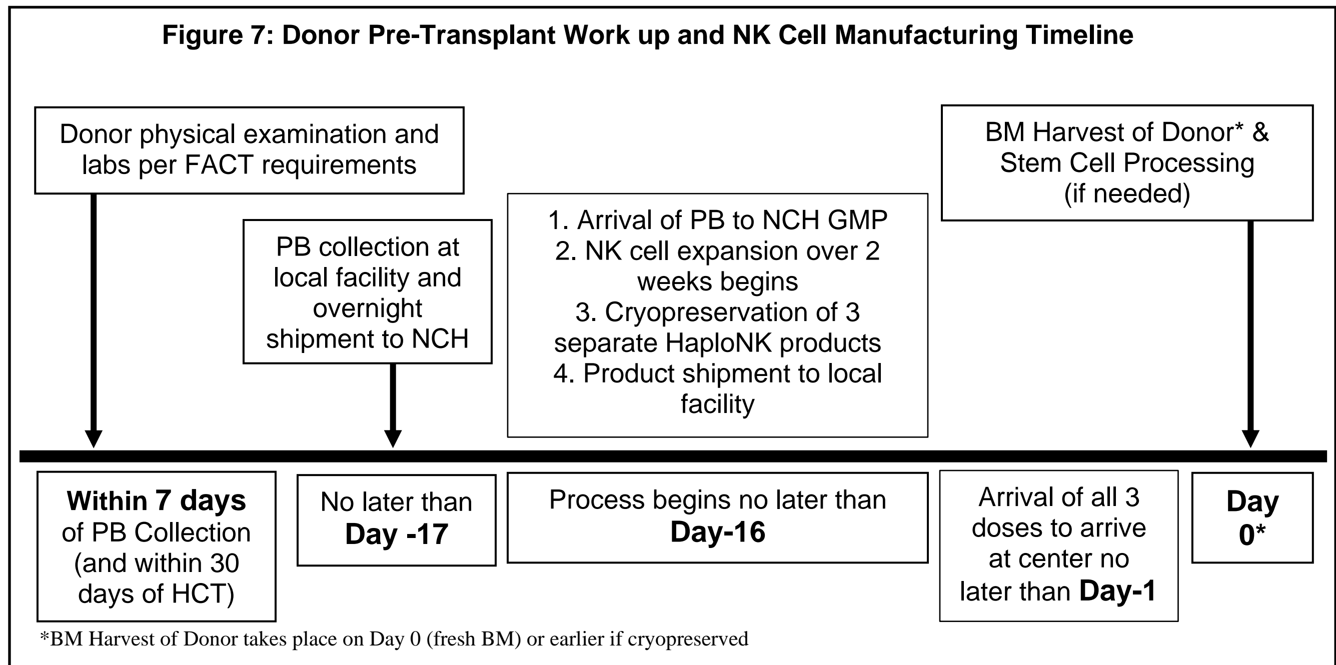
This study involves two donations **(Figure 7)**:

1. Whole blood will be drawn from the donor and collected in a sodium heparin bag. This unit will be collected to start the NK cell expansion and needs **to arrive** to Nationwide Children's Hospital (NCH) GMP facility **at least 16 days** before the scheduled stem cell infusion (Day 0). Collection of blood is dependent on donor's weigh**t.** Peripheral blood (PB) ≤ 450 mL with a minimum of 10 ml/kg

based on recipient body weight should be collected. Please see **Manual of Procedures (MOP) for additional details.**

2. After both patient and donor have provided consent, and donor has passed donor screening tests, donor will undergo harvest of stem cells.
    - Donors may be prescribed oral iron supplementation to assist in hemoglobin recovery.
    - BM is the preferred stem cell source.
    - PBSC may be allowed after discussion with the chair/protocol team for extenuating circumstances; for example, if BM harvest under general anesthesia is medically contraindicated for the donor, or if there is considerable weight disparity between donor and recipient that there is concern about attainment of an adequate stem cell dose.
    - While fresh products are preferred and prioritized, BM and PBSC cryopreserved products are allowable.

Figure 7: Donor Pre-Transplant Work up and NK Cell Manufacturing Timeline



*BM Harvest of Donor takes place on Day 0 (fresh BM) or earlier if cryopreserved

2.4.2 NK Cell Production

On or before **Day-16,** the NK product will begin production under cGMP conditions in the Cell-Based Therapy Laboratory of the Clinical Manufacturing Facility at Nationwide Children´s Hospital (NCH). The NK cell product is produced by first isolating peripheral blood mononuclear cells (PBMC) from the donor's peripheral blood. NK cells are then propagated according to the procedures outlined in the Chemistry, Manufacturing, and Control (CMC) document. Briefly, PBMC are depleted of CD3+ T-cells using magnetic beads conjugated to anti-CD3 antibodies. The resulting cells are co-cultured with irradiated mbIL21-expressing feeder cells (IFC). Cultures are maintained in media supplemented with 100 IU/mL IL-2, with replenishment of media as needed. After 7 days, the cultures are re-stimulated with IFC at a 1:1 ratio. The NK cell product will undergo lot release testing and cryopreservation and will be labeled according to ISBT 128 guidelines. After Quality Assurance testing is complete a Certificate of Analysis will be issued.

All cryopreserved doses prepared for a patient will be shipped together to the treating institution after initial release criteria are met, at three NK cell doses of 1 x 10^8/kg (+/- 20%) of recipient body weight, based on actual weight obtained at time of donor blood collection Cryopreserved NK cells are released for infusion after passing criteria specified in the IND. NCH Laboratory Services will test in-process and final product samples. The laboratory will use a system validated for detecting aerobic, anaerobic and fungal microbial species in cell therapy products, such as the BD BACTEC or Biomerieux BacT/Alert automated microbial detection system. All tests are held for 14 days or until the product becomes positive. Positive tests are then speciated and antibiotic sensitivity determined. The results of the identity of the microorganism and antibiotic sensitivity will be communicated to the physician as soon as feasible and reported to the

appropriate agencies. The first and second infusions may take place before all samples have completed 14-day microbial testing, and as such the sterility results of the NK Cell product may be released as “Negative-to-date.” The third infusion is expected to have completed 14-day microbial testing at the time of release and distribution. Final sterility testing therefore may be negative to date and may not be available at the time of infusion. Excess cells produced during expansion beyond what is required to meet the patient dose will be cryopreserved in additional back-up aliquots. Unused backup aliquots will be released to Dr. Lee's or Dr. Bonifant’s Laboratory for correlative studies if not used during the protocol treatment. Additional unused NK cells will be de-identified/anonymized for research use unrelated to the study if consent obtained. At the end of the study, any unused extra NK cells not needed for clinical or research use will be destroyed. The selected cryopreserved products for clinical use on this trial will be shipped to the study site from NCH GMP facility in a liquid nitrogen dry shipper via overnight shipping. Specific instructions will be provided to all study sites regarding the procedure for thawing and administration of the product and are also detailed in the Manual of Procedures (MOP).

2.4.3 EXCEL Trial Treatment Plan

If only 1 or 2 doses of the NK cell product are available, the following is the order of prioritization from most important to least important infusion: 1) Day +7, 2) Day +42, 3) Day -1.

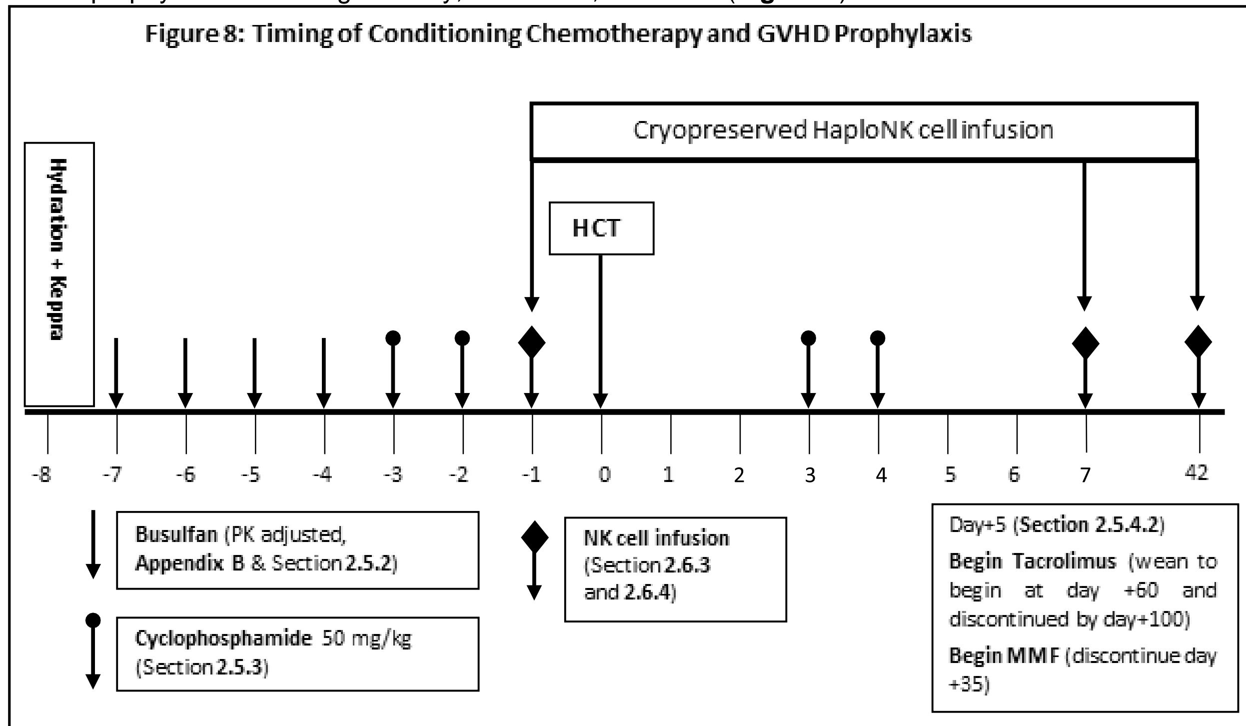
No later than Day -17	Donor blood draw and overnight shipment to NCH GMP Lab
No later than Day -16	Initiation of NK cell expansion
Day -7 through -4	Busulfan: PK adjusted to target total AUC to 65-75mgxh/L[95] (see **Appendix B** for details)
Day-3 through -2	Cyclophosphamide: 50mg/kg/day IV – recommend starting ≥15 hours from last dose of Busulfan Mesna: Per institutional standards
Day-1	First NK cell infusion[1]
Day 0	**HCT**
Day +3 & Day +4	Cyclophosphamide: 50 mg/kg/day IV Mesna: Per institutional standards
Day +5	Tacrolimus[2]: 0.015mg/kg/dose IV over 2h q 12h MMF[3]: Initiate at 15 mg/kg/dose TID (IV)
Day +7	Second NK cell infusion[4]
Day +35	MMF: Discontinue if ≤ Grade I aGVHD
Day +42	Third NK cell infusion[5]
Day +60	Tacrolimus: Begin taper if ≤ Grade I aGVHD
Day +100	Tacrolimus: Discontinue if ≤ Grade I aGVHD

[1] First NK cell infusion should be **infused at least 24hr after completion of the last dose of cyclophosphamide**.
[2] Tacrolimus may be administered as a continuous infusion, see **Section 2.5.5.2**
[3] Based upon actual weight; MMF maximum total daily dose not to exceed 3 g/day (1 g po/IV TID).
[4] Second NK cell infusion should be infused on Day+7 (± 1 day).
[5] Third NK infusion will be given on any day from Day+42 up to Day+90 after HCT and meeting infusion criteria as stated in **Section 2.6.1**. All efforts will be made to infuse NK cells as close to day +42 as possible.

2.5 Preparative Regimen and GVHD Prophylaxis

This myeloablative conditioning regimen will consist of busulfan and cyclophosphamide, with post-HCT GVHD prophylaxis consisting of PTCy, tacrolimus, and MMF (**Figure 8**).

Figure 8: Timing of Conditioning Chemotherapy and GVHD Prophylaxis



All patients will be admitted to the hospital on either day -8 (day before conditioning begins) to begin Keppra prophylaxis and IV fluids or day -7 (start of conditioning) per institution's standard practice. The patients will remain inpatient through myeloid engraftment. Patients may remain hospitalized after myeloid engraftment until sufficient resolution of any post-transplant complications per institution's standard practice. After hospital discharge, patients will be seen at least weekly in the outpatient clinic until at least 28 days following final NK cell infusion or until day 90, whichever is longer.

2.5.1 Seizure Prophylaxis

Seizure prophylaxis should be commenced according to institutional standards. Phenytoin can be used for seizure prophylaxis, however due to its interaction with busulfan, adjustments to busulfan dosage should be made on first PK to achieve target AUC. It is recommended that seizure prophylaxis be continued for a minimum of 24 hours after the last dose of busulfan.

2.5.2 IV Busulfan Administration Starting Doses

Recommended target total AUC: 65-75 mg x hour/L[94]. This translates to a CSS of 677-781 ng/mL. For AUC:

- q24 hrs AUC per dose: 16.3-18.8 mg x hour/L (3959-4568 micromolar*min)
- q6 hr AUC per dose: 4.06-4.69 mg x hour/L (990-1142 micromolar*min)

Please refer to **Appendix B** for table of conversion between different AUC values (adapted from Busulfan PK[96]). The recommended dosing interval for busulfan is q6 hourly or q24 hrs. Q12 hourly dosing is also allowed per institutional guidelines as long as the center aims to achieve acceptable AUC ranges and blood samples for PK are drawn after first dose at appropriate time intervals as per institutional standards for dose adjustments. If dose adjustments are made, it is strongly recommended that repeat PK should be performed if timing allows to demonstrate the target AUC was obtained. **It is also strongly recommended that the dose not be increased to further target within the range. For example, if the predicted total AUC will be at the lower limit of this range, we do not recommend increasing the dose to get "deeper" within the range.**

Busulfan dosing guidelines are as follows based on the following pediatric dosing nomogram by Michel et al[97]. **While the following are suggested starting doses. we recommend confirming the starting dose (especially for obesity and those below ideal weight), with the Busulfan PK Laboratory.** Contact Rosa Yeh, PharmD at ryeh@seattlecca.org, pklab@seattlecca.org, or at the PK Main Lab line (206-606-7389).

The dosing strata are:

Child's weight (kg)[1]	IV busulfan initial dose (mg/kg) based on q6hr dosing	IV busulfan initial dose (mg/kg) based on q24hr dosing
<9	0.9	3.6
9 to <16	1.1	4.4
16 to <23	1.0	4.0
23 to 34	0.85	3.4
>34	0.7	2.8

Because busulfan pharmacokinetics are linear, dosing and exposures from every 6-hour administration can also easily be extrapolated to every 12- or 24-hour dosing by multiplying by 2 or 4, respectively (the number of doses per day). For example, a 20 kg child receiving every 24-- hour dosing would receive 4.4 mg/kg for the initial dose.

[1] Busulfan dose adjustments should be considered for obesity defined as >125% of ideal body weight (IBW).

2.5.3 Cyclophosphamide, Hydration and Mesna (Mesnex®)

Cyclophosphamide (Cy)

- Cy will be given at a dose of 50 mg/kg/day IV over 1 hr x 2 days on Day -3 and Day -2.
- It is ***recommended*** to begin Cy ≥15 hours after the last dose of busulfan.
    - Cy given within 15 hours of the last dose of busulfan has been associated with significantly slower Cy clearance and longer elimination half-life, increased mucositis, and higher incidence of sinusoidal obstruction syndrome[98]
- **Dosing of Cy is based on the lesser of IBW and actual body weight. Adjusted body weight is not used in this protocol for CY dosing.**
- Hyperhydration and maintenance of significant urine output after administration is required per institutional guidelines.

Mesna (Mesnex®)

- Mesna must be administered to prevent hemorrhagic cystitis.
- Mesna dose will be based on the Cy dose being given. The dose of Mesna must be ≥ 80% of the total daily Cy dose and should be administered as per institutional standards.
- A suggested approach is as follows:
    - Mesna 10 mg/kg lesser of IBW or actual body weight/dose IV mixed with Cy over 1 hr, followed immediately by Mesna 40mg/kg lesser of IBW or actual body weight IV continuous infusion between hours 1-13 once daily.

2.5.4 Bone Marrow Processing and Infusion

On Day 0, patients will receive T-cell replete BM. If there is a major ABO incompatibility, red blood cells will be depleted from the donor BM using institutional practices. It is recommended that minor ABO incompatible grafts have plasma removed; however, institutional practices will determine the extent of processing for minor ABO incompatibilities. Donor BM will be harvested with a target yield of **4 x 10^8 nucleated cells/kg of recipient's IBW**, and a recommended minimum yield of 2.5 x 10^8 nucleated cells/kg of recipient's IBW. In addition to calculating the TNC dose/kg, a sample of the product to be infused will be sent for flow cytometry to determine the content of $CD34^+$cells. Discussion with Protocol Chair/team is necessary when PBSC are required due to extenuating circumstances. **The use of cryopreserved stem cells is allowable**

when fresh stem cells are unobtainable. During the COVID-19 pandemic surges, changes in donor guidelines have mandated use of cryopreserved products to preserve safety. Several recent studies have demonstrated that cryopreserved stem cell products have equivalent outcomes to fresh products, in particularly when using the PTCY platform[99] and in malignant diseases[100,101].

Days of rest may be added between Day -1 and Day 0 (prior to stem cell infusion) to accommodate donor availability, operating room schedules, delay in arrival of NK product etc., as clinically indicated.

2.5.5 GVHD Prophylaxis and Treatment

2.5.5.1 Post-Transplantation Cyclophosphamide (PTCy) with Mesna (Mesnex®)

PTCy will be given at a dose of 50 mg/kg/day IV over 1 hr x 2 days on Day+3 and Day+4. The first dose of PTCy on day +3 must be given between 60-72 hours from the start of the stem cell infusion. Dosing of cyclophosphamide is based on the lesser of IBW and actual body weight. Hyperhydration, maintenance of significant urine output after administration, and concurrent Mesna use is required and can be managed as per institutional guidelines. See **Section 2.5.3** for details.

No immunosuppressive agents should be given from Day -2 (24 hrs before the first NK cell infusion) until 24 hours after the completion of Day +4 PTCy, with exception of physiologic hydrocortisone. This includes corticosteroids as antiemetics. Corticosteroids should continue to be avoided even beyond this period, unless used for adrenal support, suspected GVHD, or for medical emergencies (e.g., treatment of anaphylaxis). If used, attempt to limit to <0.5 mg/kg prednisone equivalent if possible.

2.5.5.2 Tacrolimus and Mycophenolate Mofetil (MMF, CellCept®)

On Day+5, patients will begin prophylaxis with tacrolimus and mycophenolate mofetil (MMF). Begin these at least 24 hrs after the last PTCy infusion.

The starting dose of tacrolimus is 0.015mg/kg IBW/dose IV over 2 hrs every 12 hrs. Serum trough levels of tacrolimus should be measured beginning Day+7 and the dose adjusted to maintain a level of 5-15 ng/mL (or institutional equivalent). **Continuous infusion of tacrolimus per institutional standards is also allowed on this protocol.** Tacrolimus should be converted to oral dosing when patient has a stable, therapeutic level and is able to tolerate food or other oral medications. For pediatric patients, the oral dosing is approximately two to four times the IV dosing. It is recommended that serum trough levels should be checked at Css after any dose modification and when switching from IV to oral to ensure therapeutic trough concentrations. Serum trough concentrations should be checked at a minimum weekly thereafter and the dose adjusted accordingly to maintain a level of 5-15 ng/mL. **It is recommended that the tacrolimus wean begins on Day+60 if there is no clinical evidence of > Grade I aGVHD**. Tacrolimus should be weaned by approximately 25% per week beginning at Day+60 and discontinued after the last dose around Day+100. If patient has active > Grade I aGVHD this recommended taper can be modified and tacrolimus may be continued per the discretion of the treating physician or per institutional guidelines. Tacrolimus may also be discontinued early if patients relapse. This information i.e., early tacrolimus discontinuation and or continuation beyond 100 days and rationale for same should be included in the Case report forms (CRF).

Mycophenolate Mofetil (MMF, CellCept®)

MMF will be given at a dose of 15mg/kg/dose IV TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 g/day (1 g IV TID). MMF should be converted to oral dosing when patient is able to tolerate food or other oral medications. MMF prophylaxis will be discontinued after the last dose on **Day+35** unless there is concern about engraftment and/or GVHD. Information regarding duration of MMF therapy other than what is specified in the protocol i.e.(< or > 35 days) and rationale for same should be included in the CRFs.

2.5.5.3 Treatment of Active GVHD

GVHD therapies often include medications that are highly immunosuppressive to NK cells. Use thoughtfully (especially within 1 week of NK cell infusion) based on clinical necessity.

2.6 NK Cell Adoptive Transfer

2.6.1 NK Cell Infusion Criteria

Absence of ≥ Grade II GVHD or resolved GVHD but still on systemic corticosteroids ≤ 0.5 mg/kg prednisone (or equivalent). If a patient misses the third dose of NK cells due to being on treatment with high dose steroids (≥0.5 mg/kg), the patient can get the scheduled NK cell dose at a later date as long as it is within the acceptable period of administration as per protocol (Day+42 until Day+90). **Patients with unresolved/ongoing and serious viral, bacterial, or fungal infection despite appropriate treatment may still receive NK cells. This should be discussed with the Protocol Chairs prior to NK cell infusions.**

2.6.2 Verification of Patient and Product Identity

Prior to the administration of NK cells, the administration nurse and the infusing clinician will verify the identity of the recipient and the product, certifying that all of the following criteria match, and document this verification on the infusion form.

2.6.3 Timing and Dose of NK Cell Infusion

- NK cell infusions are planned on Day-1, Day+7 (± 1 day) and Day+42 (up to Day+90) following HCT.
- The first NK cell infusion **should be infused at least 24hr after completion of the last dose of cyclophosphamide**
    - In order to accommodate timing of NK cell infusion with requirement of at least 24 hrs between last dose of CY and day -1 NK cell infusion, an extra day of rest may be added.
- The second dose **should be given at least 24 hours after the last dose of PTCy.**
- The third dose can be given any time after Day+42 up to Day+90 from stem cell infusion as long as the infusion criteria as defined in **Section 2.6.1** are met.
    - To maximize the effect of this third dose of NK cells, all efforts should be made to give this dose **as close to day +42** as possible.
- If only 1 or 2 doses of the NK cell product are available, the following is the order of prioritization, from most important to least important infusion: 1) Day +7, 2) Day +42, 3) Day -1.
- NK cell content will be based on total nucleated cell (TNC) count and flow cytometry assessment of $CD56^{pos}CD3^{neg}$ percentage prior to cryopreservation.
- A fixed dose of 10^8/kg (pre-HCT weight) of NK cells/kg of actual body weight prepared at the time of cryopreservation will be administered at each time point.
- The target volume of cell product infused for each dose is 1mL/kg. Total CD3+ T cell content of the infusion will be less than 1 x 10^5/kg recipient actual body weight/dose (i.e., ≤ 0.1% of the NK cell infusion product).
- If the infusion product contains > 0.1% CD3+ T cells, the NK cell dose for infusion will be reduced in order to deliver no more than 10^5 CD3+ cells/kg recipient weight.
- If there are insufficient NK cells generated to deliver all three planned doses at 10^8/kg, then the patient may receive all cells split between Dose 1 and Dose 2 (and none at Dose 3), or all cells at the Dose 2 time point per direction of Protocol Chairs.

2.6.4 NK Cell Infusion/Administration

The following medications should be available at the bedside:

a. Diphenhydramine 1 mg/kg IV (maximum 50 mg/dose)
b. Epinephrine (1:1000) 0.01 mL/kg SQ (maximum 0.5 mL/dose)

***Administration of corticosteroids should be discussed with, and approved by, the attending physician in case of anaphylaxis. Corticosteroids will adversely affect NK cell function, so should only be given if symptoms are severe or life threatening or if other management is not effective.**

This is a cryopreserved, cellular therapy product that expires 30 minutes after thaw. Consider administration of diphenhydramine or another histamine H1 receptor blocker prior to subject receiving cryopreserved NK cells per institutional practice. Infuse cells using tubing sets per local institutional practice. In the absence

of specific guidelines, a filtered tubing set (150-260 micron) is preferred but not required. Infuse the entire thawed NK cell product and flush the bag with normal saline to ensure complete dose delivery. The NK-cell content of each dose will be based on TNC, flow cytometry assessment of CD56posCD3neg percentage, and recipient weight prior to cryopreservation

2.7 Post-Transplant Supportive Care and Relapse Prevention

2.7.1 Supportive Care

Patients will receive transfusions, nutritional support, infection prophylaxis and treatment, and other supportive care according to standard of care and institutional guidelines.

- Infection prophylaxis should include, but is not limited to, agents or strategies (e.g., PCR screening and preemptive therapy) to reduce the risk of bacterial, herpes simplex, CMV, Pneumocystis jiroveci, and fungal infections.
- G-CSF is not routinely given prior to engraftment on this protocol.
    - Reasons to give G-CSF prior to engraftment can include circumstances such as neutropenia with severe infection.
    - Other reasons for neutropenia should be considered such as Bactrim or other myelosuppressive drugs.
    - After engraftment, G-CSF may be given for severe neutropenia (ANC $<0.5 \times 10^9$/L) without discussion with the Protocol Chairs.
        - If G-CSF will be given, the dose should start at 5 mcg/kg daily.
        - Other growth factors (GM-CSF, erythropoietin) should be given only if clinically indicated.

2.7.2 Relapse Prophylaxis

Additional relapse prevention therapies (i.e., sorafenib or other tyrosine kinase inhibitors for FLT3 ITD+ AML) are allowed on this trial beginning from **Day+100** onward. Earlier requests may be considered after discussion with the Protocol Chair.

2.8 Risks and Toxicities

2.8.1 Busulfan (Busulfex®)

Busulfan (1, 4-dimethanesulfonoxybutane) is an alkylating agent. The drug is extensively metabolized by the liver and its metabolites are eventually excreted in the urine.
Toxicities:

- Gastrointestinal: Nausea, vomiting, constipation, diarrhea, abdominal discomfort, anorexia, dyspepsia, mucositis, stomatitis
- Hematological: BM ablation from which the patient would not be expected to recover without stem cell rescue
- Cardiovascular: Hypertension, hypotension and tachycardia
- Pulmonary: Dyspnea, lung fibrosis, cough, diffuse interstitial pneumonitis with fibrosis, atrophic bronchitis associated with cytologic dysplasia
- Neurological: Headache, insomnia, seizures, dizziness
- Endocrine and Metabolic: electrolyte imbalance
- Genitourinary: Amenorrhea, dysmenorrhea, testicular atrophy, gynecomastia, infertility
- Cutaneous: Erythematous skin rash, hyperpigmentation
- Hepatobiliary: Hepatic dysfunction, VOD/SOS
- Musculoskeletal: Myasthenia symptoms
- Miscellaneous: Cataracts

2.8.2 Cyclophosphamide (Cytoxan®)

Cyclophosphamide is an alkylating agent whose metabolites form cross-links with DNA resulting in cell cycle-nonspecific inhibition of DNA synthesis and function. It is activated by the liver cytochrome P450 system to cytotoxic metabolites, which form cross-links with DNA. It is cell cycle-non-specific. Commercial supply of cyclophosphamide will be used.
Toxicities:

- Gastrointestinal: Nausea, vomiting, increased AST, ALT, mucositis, diarrhea, anorexia, stomatitis, abdominal pain
- Hematological: Leukopenia, anemia, myelosuppression, thrombocytopenia, carcinogenesis
- Cardiovascular:
  - Cardiac necrosis rarely with high dose cyclophosphamide.
  - Cardiomyopathy: at doses >200mg/kg, cyclophosphamide can cause fatal myocardial necrosis with clinical heart failure. Non-specific ST changes on EKG are not unusual but a decrease in voltage is significant.
- Neurological: Headache, dizziness
- Genitourinary: Hemorrhagic cystitis (prevented by hydration and Mesna therapy or bladder irrigation), gonadal function impairment, amenorrhea, dysmenorrhea, infertility, damage to unborn baby, SIADH, hematuria
- Pulmonary: Rare pulmonary toxicity, lung fibrosis
- Cutaneous: Alopecia, urticarial rash
- Miscellaneous:
  - Teratogenic: may cause secondary neoplasms
  - Anaphylaxis (rare)
  - Fluid retention: an anti-diuretic effect is usually counteracted by furosemide administration. Careful physical examination should be made and accurate weights should be determined to detect fluid overload early.

2.8.3 Tacrolimus (FK506, Prograf®)

Tacrolimus is a macrolide immunosuppressant that inhibits lymphocytes through calcineurin inhibition.
Toxicities:

- Gastrointestinal: Constipation, diarrhea, nausea, vomiting, anorexia, bowel perforation, dyspepsia
- Hematological: Anemia, thrombocytopenia, leukopenia, thrombotic microangiopathy
- Cardiovascular: Pericardial effusion, hypertension (which may cause arrhythmia, angina, myocardial infarction)
- Pulmonary: Pleural effusion, dyspnea
- Neurological: Paresthesia, headache, tremor, encephalopathy/posterior reversible encephalopathy syndrome (PRES), dizziness, insomnia, confusion, altered mental status, seizure, blindness
- Endocrine and Metabolic: Hyperglycemia, hypomagnesemia, hypokalemia, hyperkalemia, hypophosphatemia, hyperlipidemia
- Genitourinary: Renal impairment which may require dialysis, peripheral edema
- Cutaneous: Pruritus, rash
- Hepatobiliary: Liver dysfunction
- Miscellaneous: Infection, post-transplant lymphoproliferative disorders, allergic reaction, secondary malignancy, fatigue

Drug interactions: Tacrolimus is well absorbed orally. Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system and metabolized products are excreted in the urine. Drugs that may increase tacrolimus levels include tri-azole drugs (especially voriconazole and posaconazole), nephrotoxic drugs, calcium channel blockers, cimetidine and omeprazole, metoclopramide, macrolide antibiotics, quinupristin/dalfopristin, danazol, ethinyl estradiol, methylprednisolone, and HIV protease inhibitors. Drugs that may decrease tacrolimus levels include some anticonvulsants (phenobarbital, phenytoin, carbamezepine), caspofungin, rifamycins, and St. John's Wort.

Dose adjustments: The tacrolimus dose is adjusted to maintain a serum trough level of 5-15 ng/mL. Patients with hepatic or renal insufficiency should receive doses at the lower end of therapeutic concentrations. No dose adjustments are required in patients undergoing hemodialysis.

Due to extreme interactions with voriconazole and posaconazole, the tacrolimus dose should be empirically lowered when these azoles are initiated at Css levels of tacrolimus. Guidelines are provided in **Table 1** below. Dose adjustments for therapy with other azoles may be indicated. However, the initial tacrolimus dose (on Day+5) remains fixed.

Dosing considerations with concurrent azole therapy: Triazole antifungal medications are expected to increase serum tacrolimus levels; therefor dosages of tacrolimus should be adjusted accordingly. Guidelines are provided in **Table 1** below. Of note, reversal of azole-mediated inhibition of CYP3A4 (and others) and P-glycoprotein is gradual when azoles are stopped. Therefore, immediate significant dose increases in tacrolimus are not advised when azoles are stopped. Rather, tacrolimus dose increases should be cautious and based on more frequent monitoring of levels as appropriate.

Table 1: Suggested preemptive dose reduction of tacrolimus when azoles are initiated at Css levels of tacrolimus

Antifungal	Tacrolimus Dose ↓	Comment
Voriconazole	67%	Strongly advised
Posaconazole	67%	Advised
Itraconazole	50%	Advised
Fluconazole	25%	Consider

2.8.4 Mycophenolate Mofetil (MMF, CellCept®)

MMF is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA).
Toxicities:

- Gastrointestinal: Nausea, vomiting, dyspepsia, abdominal pain, diarrhea
- Hematological: Leukopenia, thrombocytopenia, anemia
- Cardiovascular: Hypertension, hypotension, tachycardia, edema
- Pulmonary: Dyspnea, cough, interstitial lung disease
- Neurological: Headache, tremors, insomnia, dizziness, progressive multifocal leukoencephalopathy (PML)
- Endocrine and Metabolic: Hypocalcemia, hypokalemia, hyperuricemia, hyperkalemia, hypomagnesemia
- Cutaneous: Rash
- Miscellaneous: Change in vision, infection, secondary malignancy, arthralgia, myalgia, fatigue, edema, unusual contusions, allergic reaction, leg cramps

Drug interactions: MMF activity is decreased with oral antacids and cholestyramine. There are no pharmacokinetic interactions with cotrimoxazole, oral contraceptives, or cyclosporine (CSA). Acyclovir or ganciclovir blood levels may increase due to competition for tubular secretion. High doses of salicylates or other highly protein-bound drugs may increase the free fraction of MPA and exaggerate the potential for myelosuppression.

Dose adjustments: No dose adjustments are required for liver dysfunction. For renal insufficiency, MMF dosing should not be modified unless dialysis is needed, in which case MMF can be reduced to 25-50% of the starting dose.

2.8.5 Risks of NK Cell Adoptive Cell Infusions

Adverse events associated with infusion of autologous or allogeneic NK cells:

Likely (> 20% occurrence)

- Flu-like symptoms, including fever, chills, cough, dyspnea, myalgia, arthralgia, fatigue, headache.
- Cryoprotectant (DMSO) associated toxicities: unusual taste or odor, hypertension/ hypotension, tachycardia, and headache.

Less Likely (≤ 20% occurrence)

- Acute infusion reaction: fever, chills, cough, dyspnea, hypoxia, pulmonary edema, hypotension, tachycardia, angioedema, bronchospasm, rash, pruritis, myalgia, headache, hematuria, renal insufficiency.
- The above toxicities are expected to be ≤ Grade 3 events, occur in the first 24 hours after NK cell infusion, responsive to supportive care, and resolve in 72 hours.

Rare, but Serious (≤ 3% occurrence)

- Exacerbation of GVHD: Severe GVHD has not been associated with allogeneic NK cells, although exacerbation of underlying GVHD may occur in selected settings.
- GVHD may theoretically occur from contaminating T cells in the NK cell product; but, has not been described with most release limits set at ≤ 10^5/kg.
- Infection- Infection from contamination of the NK cells with bacteria is not expected. Manufacturing processes and release testing are designed to prevent this occurrence but is still possible. Infection in immunocompromised patients may be life-threatening.

These rare adverse events are possible but not expected; may be severe and may occur with delayed onset.

Adverse events associated with the anti-tumor activity of the NK cells

- Tumor lysis syndrome: may be of any grade and may depend on tumor burden at the time of treatment
- Cytokine release syndrome: occurred in only one case of the patients described above but may increase with co-administration of systemic cytokines. It is unclear whether there are settings in which CRS correlates with response, as is seen in CAR T-cells.
- Tumor inflammation: swelling and inflammation of tumors may cause neurologic symptoms or local mass effects.
- Headache, confusion, ataxia, seizures, altered mental status

3.0 SUBJECT ENROLLMENT AND EVALUATIONS

The study will be conducted in compliance with the **PTCTC Operations Manual.** Protocol team will coordinate with local PIs/regulatory teams to assist with protocol submission, approval documents, and amendment submissions. Protocol team will report results and prepare any future publications that arise from this trial.

3.1 Enrollment

The informed consent process will begin at recognition of donor and recipient subject eligibility and consent will be obtained per institutional practices. Following briefing of the research study, the coordinator or designee will provide the subject with ample time to read the Treatment Consent and will answer any of their questions regarding the document. Prior to the subject's treatment participation, the investigator or designee will ensure that the potential participant understands the research study and their role in the study. The written informed consent will be signed and dated by the subject and by the person who conducted the informed consent discussion. A signed copy of the consent form will be placed in the subject's trial chart and a photocopy will be given to the subject. Informed consent will be obtained in accordance with the Code of Federal Regulations (CFR) 21 CFR 50.25, 32 CFR 219, 45 CFR 46 and 15-2, ICH Harmonized Tripartite Guidance for Good Clinical Practice, and the Belmont Report. Also included in the consent process, Health Insurance Portability Accountability Act (HIPAA) authorization will be obtained before any study procedure is undertaken. Once signed, the Treatment Consent allows for confirming eligibility for enrollment and registering the subject. The search and determination of a suitable HLA-haploidentical donor is performed during this time.

If a subject decides to take part in the research study, their protected health information will not be shared except as allowed by law. Study personnel will maintain confidentiality of all study documents. The results of the data from the study may be published. However, the subject will not be identified by name. The subject may change their mind and withdraw from the study any time they want.

3.2 Study Evaluations and Monitoring.

Clinical studies are recommended per **Table 2** below within the parameters of the institution's policies. Studies may be performed more frequently and as clinically indicated. For details of these evaluations see text **Section 3.3** or additional sections where indicated)

Table 2: Schedule of Clinical and Research Testing and Assessments (Refer to **Section 3.3.1** for details)

Study Evaluation	**Pre-HCT *Within 4 weeks of start of conditioning***	**Pre-HCT *Within 21 days of start of conditioning***	**Day +14 ±3**	**Day +42 ±14**	**Day +56 ±14**	**Day +100 ±30**	**Day +180 ±30**	**1 year ±1 month**
CLINICAL TESTS / EVALUATIONS								
History & Physical	X							
Performance Status Evaluation	X							
HCT- Comorbidity Index Score (HCT-CI)	X							
HLA typing; anti-donor HLA antibody testing (DSA)[1]	X							
Infectious Disease Marker (IDM) Testing	X							
Pregnancy test for FCBPs[2]	X							
EKG	X							
Cardiac function: ECHO/MUGA w/ LVEF	X							X
PFTs (FEV_1, FVC, D_LCO)[3]	X							X
Chest X-Ray or Chest CT	X							
CBC & Differential[4]	X		X	X	X	X	X	X
Comprehensive Metabolic Panel (CMP)[4]	X		X	X	X	X	X	X
Chimerism analysis[5]	X			X		X		X
Bone Marrow[6]		X				X		X
CSF (only if prior history)[7]		X						
Immune Reconstitution Studies[8]						X	X	X
GVHD Assessments						X	X	X
RESEARCH TESTING								
NK correlative studies[9]			X	X	X	X		

[1] DSA: Refer to **Section 2.3.5** if positive

[2] FCBP is defined as a female of childbearing potential is defined as a female of childbearing potential as a sexually mature female// who:

1. has not undergone a hysterectomy or bilateral oophorectomy, or
2. has not been naturally postmenopausal (amenorrhea following cancer therapy does not rule out childbearing potential) for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months)

[3] Patients < 7 years of age or those who cannot perform PFTs: O_2 Sat > 92% on room air by pulse oximetry, or not on supplemental O_2 at rest

[4] CBC and CMP will be followed post-transplant per institution's standard of care

[5] Pre-HCT: DNA for primer identification. Post-HCT: PB - sorted chimerism (CD3, CD33); BM - whole marrow chimerism (only if indicated per institutional standards of care when disease restaging is done).

[6]BM aspirate (BMA) to be sent for flow cytometry/MRD, cytogenetics, and FISH (FISH only in instances of a pre-existing known chromosomal abnormality). BM biopsy only indicated if aspirate inadequate for clinical decision making.

[7]Cerebrospinal fluid (CSF) to be sent only if prior history of positive cytology.

[8] IgG, A, M, and T/B lymphocyte subsets

[9] A single research blood sample of 10mL (Sodium Heparin or Lithium Heparin tube) at each timepoint will be obtained and shipped at ambient temperature for NK cell cytotoxicity, immunophenotyping, and serum cytokine production in the Lee or Bonifant Laboratory; see **Section 3.3.1.2.3.**

3.3 Evaluations

3.3.1 Recipient

Potential subjects must meet all eligibility criteria to be considered for enrollment onto the study. All subjects will require documentation of a detailed history and physical examination and standard BMT evaluation of cardiac, pulmonary, liver and renal function. All subjects will undergo a unilateral bone marrow aspirate (and biopsy if needed) for morphological, cytogenetic and flow cytometric evaluation no later than 21 days prior to the start of conditioning.

3.3.1.1 Baseline Evaluations

1. History and Physical examination including height and weight
2. Performance status evaluation (Lansky for <16 years; Karnofsky for ≥16 years)
3. HCT-Specific Comorbidity Index score
4. HLA typing (HLA-A, -B, -C, -DRB1)
5. Anti-donor HLA specific antibody (DSA) testing: Positive anti-donor antibody is defined as a positive crossmatch test of any titer (by complement dependent cytotoxicity or flow cytometric testing) and/or the mean fluorescence intensity (MFI) of any anti-donor HLA locus (HLA-A, -B, -C, -DRB1) antibody >2000 by solid phase immunoassay. Consult with Protocol Chair/team for the clinical significance of any anti-donor antibody.
6. Infectious disease testing (NMDP Infectious Disease Markers): Per local institutional guidelines
7. Pregnancy testing for FCBP
8. Cardiac function testing: EKG and ECHO/MUGA scan including LVEF
9. Pulmonary function testing FEV_1, FVC, and D_LCO. Please note: if <7 years of age: O_2 Sat > 92% on room air by pulse oximetry, or not on supplemental O_2 at rest
10. Chest x-ray or chest CT
11. Hematologic: CBC with platelets, differential, reticulocyte count; ABO and Rh typing
12. Chemistries: Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase (AP), total bilirubin, total protein, albumin, calcium
13. Recipient baseline blood sample to the institutional chimerism lab
14. AML Disease Evaluation
    a. BM aspirate
        i. Flow cytometry
        ii. Cytogenetics
        iii. FISH (only in instances of a pre-existing chromosomal abnormality)
        iv. MRD testing: In absence of institutional local preference for MRD testing via multiparameter flow cytometry, MRD testing may be sent to:
            Hematologics, Inc.
            3161 Elliott Avenue, Suite 200
            Seattle, WA 98121
    b. BM biopsy (only if BM aspirate inadequate and/or for clinical decision making)
15. Cerebrospinal Fluid (CSF): CSF cytology (only required in instances of previous known CSF involvement)

Table 3: Calculated GFR CrCl Estimation Methods by Age

Age	Calculated GFR CrCl estimation method	Formula
≥ 18 years	Cockcroft-Gault formula (based on IBW) IBW (men)=50kg+2.3kg∗(height over 60inches) IBW (women)=45.5kg+2.3kg∗(height over 60inches)	CrCl (mL/min) = [(140 – Age) x (Weight in kg)]/(SCr x 72) (x 0.85 for females)
<18 years	Original Schwartz estimate	CrCl (mL/min/1.73m^2) = [length (cm) x k]/SCr k = 0.55 for adolescent females <18 years k = 0.7 for adolescent males <18 years

3.3.1.2 Post-Transplant Evaluations

Please see **Table 2** for details regarding post-transplant evaluations. Evaluations can be obtained more frequently as needed for clinical care.

Patients will remain inpatient through myeloid engraftment (3 consecutive days of absolute neutrophil count > 500/µl). Patients may remain hospitalized after myeloid engraftment until sufficient resolution of any post-transplant complications per institution's standard practice. After hospital discharge, patients will be seen at least weekly in the outpatient clinic until at least 28 days following final NK cell infusion or until day 90, whichever is longer.

3.3.1.2.1 GVHD Assessments

Clinical assessments are to be performed per institutional procedures using **Appendix C and Appendix D** as guides. Case Report Forms will capture data at the following timepoints: Day+100 (to capture GVHD data from days 0 to +100), +180 (to capture GVHD data from days +101 to +180), and +1 year (to capture data from +6 months to +1 year).

3.3.1.2.2 Clinical: Immune Reconstitution of T cells and B cells

PB will be collected at **Days +100, +180, and +1 Year** for T and B cell immune reconstitution as part of standard clinical testing, which will comprise of:

1. Flow cytometry for lymphocyte subsets
2. CBC with differential
3. Immunoglobulins

3.3.1.2.3 Research: NK Cell Correlative Studies

PB samples (10 ml in Sodium Heparin or Lithium Heparin tube at ambient temperature) will be collected at **Days +14, +42, +56, +100** (see **Table 2** for appropriate windows for collection) to analyze NK cell persistence, phenotype, and function, and assess plasma cytokines levels as part of research testing. Briefly, the activation of NK cells will be determined by flow cytometry-based methods determining cytokine production and CD107a expression of NK cells in response to standardized targets. The cytolytic function of NK cells will be assessed by cell lysis of standardized targets. NK cell phenotypes will be assessed using flow cytometry or mass spectrometry. Cytokines will be measured by flow cytometry-based bead array or multiplex luminometric methods. Clinical response will be correlated with NK cell persistence *in vivo*, cytokine levels, and expression of activation markers. Research specimens should be packaged as described in the study MOP.

3.3.2 Off Treatment and Off Study Criteria

3.3.2.1 Off Treatment Criteria

- Inability to infuse any of the three NK cell products (for example, due to failure to meet release criteria or insufficient cell dose (≤ 10^8 NK cells/kg actual body weight), or other manufacturing/shipping/local site issues)
- Failure to receive NK cells because of Grade ≥ 2 aGVHD or resolved GVHD but still on systemic steroids (≥ 0.5 mg/kg)
- Relapse/disease progression requiring further treatment

- Unexpected pattern of toxicity
- Patient withdrawal from study
- Patient is non-compliant with treatment schema

3.3.2.2 Off Study Criteria

- Refusal of further study follow up by patient or legal guardian
- Lost to follow up
- Death

3.4 Donor

Please see **Section 2.4.1** and **Figure 7** for additional details regarding donor timelines. Standard donor evaluations for assessing eligibility should be performed per institutional guidelines.

Table 4: Donor Evaluations and Procedures

All timeframes are in reference to Day 0 being day of subject's transplant. Please note that 1 Unit of blood (volume based on donor size, see **MOP** for details) **<u>must arrive</u> to the NCH GMP facility *<u>no later</u> than day -16.*** It is important to account for shipping time and to contact the Lee Lab once a subject is being considered for this trial in order to reserve an NK Cell manufacturing spot and appropriately time blood donation. See **MOP** for details. All tests and procedures are standard clinical donor assessments except as indicated below.

Test	Day -24*	Day -17*	Any timepoint prior to Day 0	Day 0
Infectious Disease Markers (IDM)[1]	X			
RESEARCH 1. Peripheral Blood (PB) Collection (≤ 450 mL, based on donor weight)[2] 2. NK Research Studies[3]		X		
KIR typing[4]			X	
Baseline sample to chimerism lab			X	
Bone Marrow Harvest and Infusion[5]				X

** Timing of IDMs is based on timing of blood collection. Listed in table is the latest timeframe possible for blood collection due to NK cell manufacturing/trial logistics*

[1] **Donor IDMs must be drawn, and clearance must take place *<u>within</u>* <u>7 days</u> prior to PB collection for NK cell production and *<u>within</u>* <u>30 days</u> of stem cell collection for transplant.** For example, for a blood collection happening on day -17, IDMs must be drawn *<u>no earlier</u>* than day -24. In order to send only one sample for Donor IDMs to meet clearance requirements for both PB and stem cell collection, the sample will have to be drawn no earlier than day -24. This will satisfy requirements for both collections and avoid sample expiration.
[2] Refer to MOP for blood collection guidelines. Blood (starting product for generation of NK cells) **should arrive at the NCH GMP facility *<u>no later</u>* than day -17**. Please account for shipping timeline when planning PB collection and IDMs.
[3] A single research blood sample of 10mL will be obtained and analyzed for NK cell cytotoxicity, immunophenotyping, and serum cytokine production in the Lee or Bonifant Laboratory and *will be taken from the 1 unit of PB sent to the Lee Lab*. No need to send a separate tube; see **Section 3.3.1.2.3** for details of studies.
[4] KIR typing is **<u>not required</u>** for this protocol, but if clinically obtained as part of institutional HLA-haploidentical donor selection, then report on CRF.
[5] Collection and infusion of fresh marrow is performed per institutional guidelines, which may allow a window of collection to occur within 1-3 days of Day 0.

4.0 STUDY PARAMETERS

4.1 Product Manufacturing Failure

Inability to generate sufficient NK cell product due to failure to meet release criteria or insufficient cells for at least one full dose (≤10^8/NK cells/kg actual body weight).

4.2 Neutrophil Recovery

The cumulative incidence of neutrophil engraftment from the time of transplant will be estimated using the cumulative incidence function with death and relapse prior to engraftment as the competing risk. The definition of neutrophil engraftment is a post-nadir ANC > 500/mm^3 for three consecutive laboratory values obtained on different days. The first of the three days will be designated as the day of neutrophil recovery.

4.3 Platelet Recovery

The cumulative incidence of platelet engraftment from the time of transplant will be estimated using the cumulative incidence function with death and relapse prior to engraftment as the competing risk. The definition of platelet engraftment is sustained platelet count > 20,000/mm^3 with no platelet transfusions in the preceding seven days. The first of three consecutive measurements on different days will be designated as the day of initial platelet recovery.

4.4 Donor Chimerism

Mixed donor chimerism is defined as >5%, but <95%, donor cells detected. Full donor chimerism is defined as >95% donor.

- At baseline, a sample of PB from the patient, and either harvested BM or blood from the donor, are collected for genetic studies to establish a baseline for subsequent chimerism assays.
- Post-HCT, chimerism will be evaluated by PB sorted chimerism (CD3, CD33) and BM whole marrow chimerism (only if indicated per institutional standards of care when disease restaging is done) at **Day+42, Day+100, and +1 year**.

Methods may include:

1. PCR analysis of variable number of tandem repeats (VNTR) in PBMC if informative
2. Restriction fragment length polymorphism (RFLP) if the donor and recipient RFLPs are informative
3. Fluorescence in-situ hybridization (FISH) for Y-chromosome markers on PBMC if the donor is male and patient is female
4. Cytogenetic analysis
5. Flow cytometric analysis of HLA-A, -B or -DRB1 on lymphocytes in the PB if haploidentical and suitable reagents exist
6. Other institutional standards
7. Chimerism should also be determined from the BM at required time points

4.5 Graft Failure

Primary Graft Failure

PGF is defined as failure to achieve an ANC ≥ 0.5 x10^9/L for 3 consecutive days by Day+42 and < 5% donor-derived cells by BM or PB chimerism studies and no evidence of persistent or relapsing disease.

Secondary Graft Failure/Late Rejection

Secondary graft failure/late rejection is defined as initial donor-derived neutrophil engraftment followed by subsequent decline in ANC to <0.5 x10^9/L with <5% donor-derived cells by BM or PB chimerism, unresponsive to growth factor therapy, unexplained by disease relapse or medications. Patients who have late rejection of the donor graft with autologous reconstitution of recipient BM should also be considered secondary graft failure. Assessment for this endpoint will occur up to 1-year post HCT.

4.6 Graft Versus Host Disease

4.6.1 Acute GVHD

The cumulative incidence of aGVHD Grades II-IV and III-IV will be assessed according to standard assessment criteria stated in **Appendix C.** It is strongly recommended, when possible (not required), that

biopsies be taken for histological confirmation of GVHD of an affected organ (e.g., skin, liver, or gastrointestinal tract). Date of symptom onset, date of biopsy confirmation of GVHD, maximum clinical grade, sites affected, and dates and types of treatment will be recorded. Dates of symptom onset of Grade II or higher GVHD and Grade III-IV GVHD will be recorded. The cumulative incidences of acute Grade II-IV and Grade III-IV GVHD will be determined through competing risk analysis. Relapse/progression, graft failure, or death without aGVHD are considered competing risks for aGVHD. In addition, aGVHD will be reported with only graft failure and death regarded as competing risks. Recommended primary systemic treatment for acute GVHD will be corticosteroids. However, initiation of calcineurin inhibitors or other systemic immunosuppressants will be per discretion of the treating physician. All efforts should be made to record the systemic immunosuppressants used, timing of their administration and duration of treatment administered beyond the originally planned prophylaxis regimen. We will characterize the duration, number, and type of steroid and non-steroid immunosuppressants used to treat aGVHD.

4.6.2 Chronic GVHD

The cumulative incidence and severity of cGVHD will be assessed according to **Appendix D** using the NIH consensus criteria[102]. Date of onset, date of biopsy confirmation (if any), dates and types of treatment, and extent will be recorded. The cumulative incidence of chronic GVHD (overall and according to extent) will be determined through competing risk analysis. Relapse/disease progression, graft failure, or death without cGVHD are considered competing risks for cGVHD. The cumulative incidence of cGVHD will be described at Day+180 and +1 year. Use of systemic immunosuppressive therapy for treatment of cGVHD is at the discretion of the treating physicians. The event of interest is the development of any cGVHD severe enough to warrant systemic therapy, which includes corticosteroids (prednisone dose ≥0.5mg/kg/day or equivalent), any systemic immunosuppressive agent or extracorporeal photopheresis. Use of topical immunosuppressive agents is not necessary for triggering this endpoint. Patients who continue on immunosuppressive therapy beyond Day+180 due to manifestation of cGVHD will also be considered an event for the primary endpoint. We will characterize the duration, number, and type of steroid and non-steroid immunosuppressants used to treat cGVHD.

4.7 Disease and Survival Endpoints

4.7.1 PFS

Interval from Day 0 to date of first objective disease progression or relapse, death from any cause, or last patient evaluation. Patients who have not progressed or died will be censored at the last date they were assessed and deemed free of relapse or disease progression. Disease persistence in the absence of progression is not included in this analysis.

4.7.2 EFS

Interval from Day 0 to date of first objective disease progression or relapse, an unplanned therapeutic maneuver for disease persistence, death from any cause, or last patient evaluation. Patients without events will be censored at the last date they were assessed and deemed event-free. EFS will be estimated using the Kaplan-Meier method and EFS at +1 year and +2 years will be estimated along with a 90% confidence interval (CI).

4.7.3 RFS

Relapse free survival is the time from date of transplant to death or relapse/disease progression, whichever comes first. The event for this endpoint is relapse/disease progression or death. Patients alive and disease free will be censored at last follow-up.

4.7.4 OS

Interval from Day 0 to date of death from any cause or last patient contact. OS will be estimated using the Kaplan-Meier method. OS at +1 year and +2 years will be estimated along with a 90% CI.

4.7.5 GRFS

An event for this time to event outcome is defined as Grade III-IV aGVHD, cGVHD requiring systemic immunosuppressive treatment, relapse or disease progression, or death by any cause. Patients will be followed up for at least one year for this endpoint.

4.7.6 NRM
The cumulative incidence of death without evidence of disease progression or relapse will be characterized at Days+100, +180, and +1 year. An event for this endpoint is death without evidence of disease progression or recurrence. Relapse/disease progression is a competing risk for NRM.

4.7.7 Relapse/Disease Progression
Relapse is defined by either morphological or cytogenetic evidence of acute leukemia consistent with pre-transplant features. High clinical suspicion of relapse will most likely lead to a disease-specific evaluation, for example a BM aspirate. Cytogenetic studies or decreasing donor chimerism also increase the suspicion of relapse as well and can sometimes detect asymptomatic relapse in routine protocol-related BM samples. If there is evidence of early relapse and no evidence of GVHD following HCT, immunosuppressive therapy may be discontinued earlier than indicated in **Section 2.5.5**. and CRF forms should be updated and the Protocol Chairs should be notified of the relapse. If there is evidence of decreasing donor chimerism and no evidence of GVHD, patients may be eligible for subsequent donor lymphocyte infusions (DLI)/

Relapse will be diagnosed when at least one of the following is present:
- Reappearance of leukemia blast cells in PB; or,
- >5% blasts in BM, not attributable to another cause (e.g., BM regeneration)
- The development of extramedullary leukemia or leukemic cells in the CSF
- The reappearance of cytogenetic abnormalities present prior to HCT

Designation of disease status in other histologies will also follow standard criteria. NRM is a competing risk for relapse/disease progression.

Institution of any therapy to treat persistent, relapse/disease progression, including the withdrawal of immunosuppressive therapy or DCI, will be considered evidence of relapse/disease progression regardless of whether the criteria described above were met.

4.8 Minimal Residual Disease (MRD)
MRD is defined as evidence of malignant cells by flow cytometry (≥ 0.1%), in the absence of morphological or cytogenetic evidence of disease in PB or BM. Since the frequency and sensitivity of testing for MRD are variable, evidence of MRD will not be sufficient to meet the definition of relapse or disease progression in this study but will be captured in the case report forms (CRFs) along with data on changing management in response to MRD detection.

4.9 Primary Cause of Death
Primary cause of death will be classified as follows:
- **Relapse/Disease progression:** Death from relapse/disease progression after Day 0, even in instance where a patient subsequently developed GVHD, organ toxicities or infections that may have contributed to subsequent death
- **GVHD:** Death from acute or cGVHD, in the absence of relapse/disease progression
- **Infection:** Death from documented viral, bacterial or fungal infections in the absence of GVHD or relapse/disease progression
- **Organ toxicity:** Death from major organ toxicities not attributable to AML, infection or GVHD
- **Other:** Any other causes of death than those listed above

4.10 RBC Independence
Red blood cell independence will be classified as follows:
- Recipient did not require pRBC transfusion in the preceding 28 days.
    - If recipient received an RBC transfusion for a procedure and is otherwise transfusion independent, report recipient as RBC independent.
- Evaluations will occur at Day+100 and +1 year.

5.0 STATISTICAL CONSIDERATIONS

5.1 Study Design Synopsis/Overall Study Design

This is a Phase II pilot study designed to estimate the 1-year RFS survival of pediatric patients with high-risk AML after haploHCT with PTCy and donor NK cell infusions, assess feasibility of product manufacturing and administration, and compare efficacy to historical controls.

5.2 Accrual and Sample Size Considerations

A BMT CTN trial of haploHCT with PTCy reported a 1-year relapse rate of 45% with NRM of 7% in 50 patients undergoing haploHCT (personal communication S. Ciurea). Adults under age 55 with AML were reported to have a 41% 1-year relapse rate after haploHCT with PTCy, and the pediatric data from Jacobsohn[18] suggests a 1-year relapse rate of approximately 35% for MRD- pediatric AML patients receiving an allogeneic HCT. Therefore, we will conservatively assume a baseline 1-year DFS of at least 65%.

In the Phase II clinical trial of this approach conducted in adults at MD Anderson, RFS improved by 30% (from 55% to 85%) with the addition of donor NK cell infusions compared to a matched cohort of similar patients receiving haploHCT with PTCy[44]. Two additional 4:1 case-matched cohorts of patients receiving haploHCT with PTCy were identified through the CIBMTR patient database, comprised of those receiving MAC or RIC regimens, respectively. 1-year RFS in the RIC and MAC arms were similar to the MD Anderson comparison cohorts at 49%, and 65% respectively. The ongoing Phase II study of haploHCT with PTCy and donor NK cell infusions in adults currently reports 1-year relapse of 4% [82] (1 of 24), and this study utilizes a RIC regimen. We use the 1-year RFS to drive the sample size. With 30 patients, the margin of error for the exact binomial 95% CI for 1-year RFS would be approximately ±12.8% (assuming a "true" RFS of 85%), with a maximum possible margin of error of, at most, ±17.9 (assuming a "true" RFS of 50%) (**Table 5**).

Table 5: Margin of Error for 95% CI from Varying Sample Sizes

Sample Size	Estimated 95% CI Margin of Error for RFS	Maximum Margin of Error for 95% CI
15	±18.1%	±25.3%
20	±15.6%	±21.9%
25	±14.0%	±19.6%
30	**±12.8%**	**±17.9%**
35	±11.8%	±16.6%
40	±11.1%	±15.5%
45	±10.4%	±14.6%
50	±9.9%	±13.9%

Outcomes for the pediatric patient population receiving this regimen are not well-described, so this study is designed to perform a preliminary assessment of efficacy for the addition of NK cell infusions to a known transplant regimen. Patients must receive a minimum of one NK cell infusion on day + 7 (Dose 2 time point) at a dose of at least 10^8 NK cells/kg actual body weight to be evaluable for RFS. For increased statistical rigor after the study is completed, will compare results from this trial with a dataset of matched controls. Thus, we entertain the 1-sided hypothesis that NK cell infusions will improve the 1-year RFS of children with high-risk AML receiving haploHCT with PTCy by at least 20%, from a baseline of 65%. The matched cohort will comprise of pediatric AML patients receiving haploHCT with PTCy selected from the CIBMTR database – matching will be done on the basis of age, sex and clinical remission status. In **Table 6** below, we have selected a sample size of 30, having sufficient power (80%) to detect an improvement in 1-year RFS from 65% to at least 85% employing a 3:1 matched cohort. This comparison was modeled for a variety of potential sample sizes and matching scenarios. All designs are 1-sided and employ a 10% Type-I error rate and an 80% power to detect an increase from a baseline 1-year RFS of 65%.

Table 6: Modeling for Trial Sample Size (n)

n	Historical control 1-year RFS	1:1 matching	2:1 matching	3:1 matching
15	65%	95.1%	92.1%	90.9%
20	65%	92.0%	89.2%	88.1%
25	65%	89.7%	87.1%	86.0%
30	**65%**	**87.9%**	**85.4%**	**84.4%**
35	65%	86.5%	84.1%	83.1%
40	65%	85.3%	83.0%	82.1%
45	65%	84.3%	82.1%	81.2%
50	65%	83.4%	81.3%	80.4%

*All designs are 1-sided and with a 10% Type-I Error Rate **80% CI. The table-specific calculations (i.e., variation of sample sizes with matching ratios to have 80% power to detect) were obtained using 1-sided Z tests with 10% Type I error. Calculations were performed using G*Power software, version 3.1.7.

5.3 Analysis of Primary Endpoints

5.3.1 1-Year Relapse-Free Survival (RFS)

1-year RFS will be estimated by the proportion and corresponding 95% exact binomial CI of patients who are relapse-free at 1-year from day of transplant (Day 0). Kaplan-Meier estimate of 1-year RFS will be used if 1 year follow up has not been reached for all surviving patients.

Sensitivity analysis in patients who receive relapse prevention therapies (e.g., FLT3 inhibitors) post haploHCT: In the event that there are enrolled patients who receive potential relapse-preventing therapies we will perform calculate survival estimates and perform sub-analyses of those who did and did not receive these therapies.

5.4 Safety Endpoints and Stopping Rules

We will perform continuous monitoring of the following safety endpoints.
The study will be held for review by DSMC if it appears that rates of these events (determined by exact one-sided binomial probabilities) significantly surpass their respective percentage:

- Grade III-IV aGVHD (**Table 7)**): 25%
- Graft failure (**Table 8**): 15%
    - Graft failure attributed to a low cell dose of the infused graft will not be counted for the purpose of calculation for the stopping rule.
- Day 100 TRM (**Table 9**): 10%
- Day 100 infections (**Table 10**): 15%
- Product Manufacture Failures (**Table 11**): 25%

If the rate of events significantly exceeds the acceptable rates, as determined by p-value <0.1, then the trial will be suspended pending DSMC review. For example, if 10 patients have been enrolled and treated at the time of the second observed graft failure, then the stopping criteria would be triggered due to there being evidence that the graft failure rate may be significantly greater than 20%. However, if more than 10 patients have been enrolled and treated at the time of the second graft failure, then the stopping criteria has not been met and the trial may continue.

Table 7: Monitoring of Grade III-IV aGVHD

≥Grade III GVHD	Suspend Study if n enrolled ≤	GVHD Rate
1	-	-
2	-	-
3	4	75%
4	7	57%
5	10	50%
6	13	46%
7	16	44%
8	19	42%
9	23	39%
10	26	38%
11	29	38%
12	33	36%
13	36	36%

Table 8: Monitoring of Graft Failure

Graft Failures	Suspend Study if n enrolled ≤	Failure Rate
1	-	-
2	3	67%
3	7	43%
4	12	33%
5	17	29%
6	22	27%
7	27	26%
8	30	27%

Table 9: Monitoring of Day 100 Transplant Related Mortality

Number of TRM by day +100	Suspend Study if number of total enrolled patients is at or fewer than:	TRM Rate
1	-	-
2	5	40%
3	11	27%
4	18	22%
5	25	20%
6	30	20%

Table 10: Monitoring of Day 100 Grade 4-5 Infections

Grade 4-5 infections by day +100	Suspend Study if number of total enrolled patients is at or fewer than:	Infection Rate
1	-	-
2	3	67%
3	7	43%
4	12	33%
5	17	29%
6	22	27%
7	27	26%
8	30	27%

Table 11: Product Manufacturing Failure

Product Manufacture Failures	Suspend Study if n enrolled ≤	Failure Rate
1	-	-
2	-	-
3	4	75%
4	7	57%
5	10	50%
6	13	46%
7	16	44%
8	19	42%
9	23	39%
10	26	38%
11	29	38%
12	33	36%
13	36	36%

To monitor other unforeseen toxicities and ensure adequate safety monitoring in the initial subjects, staggering will be applied to first 5 subjects. The second subject will be enrolled only after the first subject has finished 30 days of safety monitoring from the first NK infusion with no unexpected toxicities, and the third subject will be enrolled only after the second subject has finished 30 days of safety monitoring from the first NK infusion with no unexpected toxicities. After the third subject finishes 30 days of safety monitoring from the first NK infusion with no unexpected toxicities, the next 2 subjects may be enrolled within 30 days of each other. After those 2 subjects finish 30 days of follow-up with no unexpected toxicities, enrollment may proceed freely for the remaining subjects. If a patient experiences a Grade 5 toxicity that is at least probably or directly related to *ex-vivo* expanded NK cell product, we will hold all infusions on the study until both the incident in question and the infused product have undergone a thorough internal review and the outcome discussed with the FDA.

5.5 Analysis of treatment feasibility:

The analysis of treatment feasibility will be descriptive. Feasibility is defined by Product Manufacture Failure; the inability to infuse the NK cell product due to product contamination or insufficient cell dose (anything below x 10^7/kg). The percentage of patients who are able to successfully receive all three NK infusions will be recorded as well as the timing between patient referral and treatment. The total number of NK cells in the final expansion product will be recorded and the distribution of those numbers with medians and other percentiles summarized. We will record the percent of patients registered who (a) received at least one dose of NK cells at the intended dose, (b) those who received all doses of NK cells at the intended dose (c) the number of patients for which all doses of NK cells were available at the intended dose.

5.6 Analysis of Secondary Endpoints

5.6.1.1 To determine the safety and feasibility of manufacturing and administering donor-derived *ex-vivo* expanded NK cells in children and adolescents with high-risk AML receiving HLA-haploHCT.

5.6.1.2 To quantitate and characterize NK- and T-cell immune reconstitution and function following HLA-haploHCT and donor-derived *ex-vivo* expanded NK cells at Days+30, +100, +180, and +1 year, descriptive statistics will be used.

5.6.1.3 To estimate the incidence of aGVHD (Day+100) and cGVHD (Day+180, +1 year), infections (Days +100, +180), and OS (+1 year and +2 year), proportions and corresponding 95% CI will be used. OS estimates will be estimated using the Kaplan-Meier method.

5.6.1.4 To estimate the incidence of mixed donor chimerism (Days +42, +100, +1 year).

5.6.1.5 Frequencies will be tabulated to determine the presence of KIR ligand-ligand mismatch between HLA-haploidentical donor and host. The impact of this mismatch on relapse rate may be explored using chi-square or Fisher's Exact test, if applicable.

5.6.1.6 Descriptive statistics will be used to characterize the phenotype and function of the donor-derived *ex-vivo* expanded NK cell product.

5.7 Analysis of Efficacy

Efficacy of NK cells will be analyzed by comparing 1-year RFS from this prospective trial with matched controls taken from the CIBMTR database using a two-sample Z-Test. RFS and OS well be summarized using the Kaplan-Meier method and comparisons made using log-rank tests. Cox regression models will be used to assess the impact of treatment on survival while controlling for underlying demographic and disease-specific characteristics. P-values less than 0.10 will be considered statistically significant.

5.8 Data Analysis

All adverse events will be tabulated by dose and the fitted dose-toxicity curve will be summarized. The distributions of time-to-event outcomes, including RFS, OS, and times to platelet and neutrophil engraftment will be estimated separately by dose using the Kaplan Meier method and distributions will be compared using log rank tests.

6.0 REPORTING REQUIREMENTS

General guidelines: This study will be conducted in compliance with the PTCTC-approved EXCEL Study Manual of Operations. The protocol chair will serve as a liaison and will coordinate protocol development, submission, approval, amendments, results reporting and publications.

6.1 Registration

Register all patients with the PTCTC Operations Center Coordinator. Study Registration procedures are described in the EXCEL Study Manual of Procedures (MOP).
Prior to protocol enrollment and initiation of treatment, subjects/legal guardians must sign and date an IRB-approved consent form. Patients having documented consent and meeting all eligibility criteria will be enrolled in the study and assigned a unique patient study identification number by the local site.

After eligibility screening and local registration, patients who are selected to participate will be registered with the PTCTC Operations Center coordinator. For each eligible patient, a copy of the signed patient consent form, registration form, eligibility checklist, and copies of all source documentation of all clinical studies confirming eligibility and HLA typing results will be kept locally. A record of patients who fail to meet entry criteria (i.e., screen failures) will be maintained by each individual site. Patient registration must be complete before beginning any treatment or study activities.

Study Registration procedures are described in the EXCEL Study Manual of Procedures (MOP). Patients should be registered at least one month before start of conditioning regimen. Following registration, patients should begin protocol treatment within two weeks. Issues that would cause treatment delays should be discussed with the Principal Investigator. The Study Coordinator should be notified of cancellations as soon as possible.

Institutional Registration

Patient registration at each study site/institution will be conducted according to the institution's established policies. Before registration, patients will be asked to sign and date an Institutional Review Board (IRB)-approved consent form and a research authorization/HIPAA form. Patients must be registered with their local site/institution and also with the sponsor before beginning any treatment or study activities.

6.1.1 Data Reporting and Regulatory Requirements

Principal Investigator/Protocol Chair is responsible for performing the following tasks:

- Coordinating development of the protocol as well as its subsequent amendments
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study

- Reviewing and ensuring reporting of Serious Adverse Events (SAE)
- Reviewing data from all sites

PTCTC is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site
- Establishing procedures for documentation, reporting, and submitting of AEs and SAEs to the Protocol Chair, and all applicable parties
- Facilitating audits by securing selected source documents and research records from participating sites, or by auditing at participating sites
- Ensuring that all participating Transplant Centers are using the correct version of the protocol
- Ensuring that each participating institution has a valid FWA number
- Ensuring that participating sites are accruing a representative sample consistent with the estimated population of the site
- Preparing all submitted data for review by the Protocol Chair

Participating Sites are responsible for performing the following tasks:

- Securing IRB approval of the protocol and all subsequent amendments.
- Implementing and adhering to the guidelines of Good Clinical Practice (GCP)
- Submitting data to the PTCTC
- Registering all patients and maintaining the patient registration form, eligibility checklist, and signed informed consent.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol
- Maintaining regulatory binders on site and providing copies of all required documents to the PTCTC Operations Center Coordinator
- Collecting and submitting data, including the reporting of all adverse events and serious adverse events, in accordance with the schedule specified in the protocol.
- Following the protocol as written
- Verifying the current active version of the protocol with the PTCTC Operations Center Coordinator

6.1.2 Data Entry

Data collected during this study will be entered into a secure electronic data capture (EDC) system, Medidata Rave®.

6.1.3 Case Report Forms

CRFs will be generated by the PTCTC Transplant Center for the collection of all study data. Transplant Center Principal Investigators will be responsible for ensuring that the CRFs are kept up-to-date.

6.1.4 Source Documents

Study personnel will record clinical data in each patient's source documents (i.e., the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with source documents.

6.1.5 Data Submission

All data will be collected on CRFs. CRFs will be provided to participating sites by the PTCTC. A primary research data file (research chart) will be maintained at each site and must include copies of required source documentation.

6.1.6 Record Retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will

maintain all source documents, study-related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 7 years after the completion of all study activities.

6.1.7 Data Management

Research Program Coordinators

A Lead Research Program Coordinator at the PTCTC will be assigned to the study and will manage the study activities at each of the participating sites. The responsibilities of the Research Program Coordinator include project compliance, data collection, data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol team.

Study Monitoring and Quality Assurance

Regularly scheduled registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the Principal Investigator for discussion and action.

Random-sample data quality monitoring will be conducted by the PTCTC at least every 6 months; and protocol compliance audits will be conducted by the PTCTC at least once a year and more frequently if indicated. Audits by the PTCTC may entail (1) faxing source documents and research records for selected patients from participating sites to the PTCTC Study Coordinating Center for audit, or (2) on-site auditing of selected patient records at participating sites.

All clinical work conducted under this protocol is subject to Good Clinical Practice (GCP) guidelines. This includes inspection of study-related records by the PTCTC Study Coordinating Center, sponsor, its designee, or health authority representatives at any time.

6.2 Drug Toxicity and/or Adverse Reactions

6.2.1 Adverse events

Adverse events will be collected as per PTCTC standard protocols. Data on adverse experiences/toxicities regardless of seriousness must be collected for documentation purposes until 28 days after the last NK cell infusion. The date will reflect the onset and resolution date and maximum grade.

6.2.2 Unexpected serious adverse events

Unexpected serious adverse events will be collected and reported as per PTCTC standard protocols until 28 days - after the last NK cell infusion.

6.2.3 Intermittent events

Intermittent events should be labeled as such and followed until resolution.

6.2.4 If a patient is taken off study

while an event is still ongoing this will be followed until resolution unless another therapy is initiated. Pre-existing medical conditions will be recorded only if exacerbations occur during active treatment period.

6.3 Safety Analysis of Adverse Events Data

All patients who received NK-cell infusions will be included in the safety analysis. Safety and toxicity outcomes will be summarized by dose levels. Adverse event data and corresponding toxicity grades 28 days after last NK cell-infusions during long-term follow up will be summarized in the form of tables. Incidence tables will be generated to summarize incidence of patients reporting at least one episode of each specific adverse event, incidence of adverse events causing withdrawal and incidence of serious

adverse events. The total number of episodes for each event reported, the severity and attribution to study therapy of each episode reported will also be displayed.

Listings of adverse events by patients will include the time to onset, the duration of each event, the severity of each event, and the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal. Safety data will be summarized for the overall patient group and by dose levels.

6.4 Adverse Events

An adverse event (AE) is any untoward medical occurrence regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.

An AE includes, but is not limited to:

1. Any clinically significant worsening of a pre-existing condition, e.g., resulting in change of ≥1 point on the CTCAE grading scale.
2. An AE occurring from overdose (i.e., a dose higher than that prescribed by a healthcare professional for clinical reasons) of investigational product whether accidental or intentional.
3. An AE occurring from abuse (e.g., use of non-clinical reasons) of investigational product.
4. An AE that has been associated with the discontinuation of investigational product.

An AE does **not** include:

1. Medical or surgical procedures themselves (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure are AEs.
2. Pre-existing diseases or conditions present or detected prior to start of investigational product administration that do not worsen.
3. Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions).
4. Death: regarding an AE, death is an **outcome** of the AE and is not an AE itself.

GVHD and infection are collected in the EDC separately because they are endpoints. Events of GVHD and infection are not to be reported as AEs for this study unless they meet one or more of the SAE criteria noted below.

RFS is the primary efficacy endpoint and will not be reported as an AE/SAE unless it is the cause of a death that occurs between the first infusion through 28 days after the last infusion.

6.4.1 Serious Adverse Events

An adverse event is defined as a serious adverse event (SAE) when the AE

1) Results in death
2) Is considered life-threatening
3) Results in hospitalization or cause the prolongation of hospitalization
4) Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) Leads to a congenital anomaly
6) Represents a significant medical condition which, without urgent medical intervention, would lead to one of the above outcomes

Life-threatening means that the AE represented an immediate threat of death without medical intervention. It is anticipated that most if not all patients on this study will be hospitalized during the course of their allogeneic HCT. Therefore, regarding the hospitalization criterion for seriousness, only AEs that clearly result in prolongation of hospitalization should be considered serious for this study.

Clarification on SAEs:

1. All deaths, regardless of cause, must be reported for subjects on study and for deaths occurring within 30 days of last study evaluation, whichever is longer.

2. “Immediately life-threatening” means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death if it had occurred with greater severity.
3. Complications that occur during hospitalizations are AEs. If a complication prolongs hospitalization, it is an SAE. Note that the hospital prolongation should be clearly and directly attributable to the event.
4. “Inpatient hospitalization” means the subject has been formally admitted to a hospital for medical reasons, for any length of time. This may or may not be overnight. This does not refer to evaluation in an Emergency Department without admission to the hospital. This does not include planned inpatient hospitalizations for transplant or other elective procedures.
5. Patients undergoing HCT are frequently hospitalized after the initial transplant hospitalization. Subsequent hospitalizations for reasons that meet criteria for SAE or AESI should be reported as SAE. Subsequent hospitalizations for reasons that do not meet criteria for SAE or AESI need not in and of themselves be reported as SAE.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms.

6.4.2 Adverse Events of Special Interest

Adverse events of special interest (AESIs) are required to be reported by the investigator to the PTCTC Study Coordinating Center within 3 business days of knowledge of the event.

Adverse events of special interest for this study are as follows:

- Infusion related reaction including allergic reactions and anaphylaxis occurring during or post investigational product infusion.
- Cytokine release syndrome occurring during or post investigational product infusion.
- Suspected transmission of an infectious agent by the study treatment.

6.4.3 Unexpected Adverse Events

Unexpected AEs are those events the nature of which, severity, or frequency are not consistent with the known or foreseeable risk of adverse events associated with the research procedures described in the informed consent document.

6.4.4 Other Adverse Events

Other AEs will be identified by the Protocol Chairs during the evaluation of safety data. Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from the study, will be classified as other AEs. For each, a narrative may be written and included in the clinical study report.

Unanticipated problems include unexpected AEs and also unexpected problems, events, or new information which are not AEs but which indicate that research participants or others are at greater risk of harm than previously believed prior to recognition of the unanticipated problem.

6.4.5 Relationship to Treatment

Attribution of the event to the investigational product (*ex-vivo* expanded NK cells) may be characterized as follows:

- **Definitely related:** The adverse event is clearly related to the study intervention. This is most straightforward when events previously associated with the intervention (e.g., veno-occlusive disease) occur. This is less clear for infections, as this population has inherent immune deficiency prior to the intervention and may have undetected infections or active infections when the intervention begins.
- **Probably related:** The adverse event is likely related to the study intervention. The adverse event is not likely to be caused by the subject’s underlying medical condition or other concomitant

therapy, and the nature of the adverse event or the temporal relationship administration leads the investigator to believe that there is a reasonable chance of causal relationship.

- **Possibly related:** The adverse event may be related to the study intervention. The adverse event could also be attributed to the subject's underlying medical condition or other concomitant therapy, but the timing of the onset of the adverse event and study intervention leads the investigator to believe that there might be a causal relationship.
- **Unlikely related:** The adverse event is probably not related to the study intervention and an alternative explanation is more likely.
- **Not related:** The adverse event is clearly NOT related to the study intervention. The adverse event is most plausibly explained by the subject's underlying medical condition or other concomitant therapy, or the adverse event has no plausible biological relationship to study intervention.

6.4.6 Severity Assessment

The severity refers to the intensity of the AE. The Investigator must categorize the severity of each AE according to the NCI CTCAE version 5.0. CTCAE guidelines may be referenced by visiting the Cancer Therapy Evaluation Program (CTEP) page on the NCI website. For any term that is not specifically listed in the CTCAE scale, intensity will be assigned a Grade of 1-5 using the following CTCAE guidelines:

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or life-threatening. The criteria below may be used for any symptom not included in the grading scale.

<u>**SAEs (Table 10):**</u>

- Any AE ≥ Grade 4 whether attributed to NK cell infusion
- Any AE ≥ Grade 3 related to NK cell infusion

Table 10: Severity and Description of SAEs

Severity	Grade	Description	Relation to study drug/intervention
Mild	1	Does not interfere with routine activities: Minimal level of discomfort	
Moderate	2	Interferes with routine activities: Moderate level of discomfort	• Unexpected • Related to NK infusion • Within 28 days of infusion
Severe	3	Unable to perform routine activities: Significant level of discomfort	• Expected • Related to NK infusion • Within 28 days of infusion
Life-Threatening	4	Hospitalization/ER visit: potentially life-threatening	• Any timepoint • Any expectedness • Any relation to NK infusion
Death	5	Death related to AE: Any expectedness and any relation to NK cell infusion	• Any timepoint • Any expectedness • Any relation to NK infusion

FDA guidelines for toxicity will be followed; however, if a subject is evaluated in an emergency room for non-life-threatening illness or symptoms (i.e., visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed and severity of the adverse event will be assessed according to the subject's clinical signs and symptoms.

As defined by the ICH guideline for GCP, the term "severe" is often used to describe intensity (severity) of 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 subject/event outcome or action criteria usually associated with events that pose a threat to a

subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

7.0 STUDY INTERPRETATION

7.1 Aim to collect information

Since this is a Phase II pilot study, the primary aim will be to collect information about the safety, feasibility of infusing haploidentical NK cells in children and young adults with high-risk AML undergoing haploHCT with PTCy. As secondary endpoints, we will evaluate efficacy of the intervention as determined by the disease relapse rates, overall survival and incidence of GVHD.

7.2 Records to be Kept

The secure EDC system, Medidata Rave®, will contain CRFs that will document the dates and doses of therapy as well as clinical chemistries and hematologic parameters. The clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

- Imaging reports
- Surgical summaries
- Autopsy summaries, where appropriate
- Informed consent documents

All required clinical evaluation records will be the responsibility of Principal Investigator who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of immunological efficacy will be the responsibility of Principal Investigator.

7.3 Reporting Requirements

The CTEP active version (version 5.0) of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTCAE guidelines may be referenced by visiting the CTEP page on the NCI website.

- Toxicity Grading: The criteria listed in the CTEP active version (version 5.0) of the NCI Common Toxicity Criteria Scale will be used in grading toxicity.
- aGVHD will be graded by the method of Przepiorka et al [103]. See **Appendix C**
- Should unanticipated toxicities arise (e.g., Grade III-IV local reactions or hepatorenal damage) they, too, will be graded by the CTEP active version (Version 5.0) of the NCI CTCAE
- AEs will be collected as per PTCTC standard protocols.
    - Data on all adverse events regardless of seriousness must be collected for documentation purposes only for 28 days after the last NK cell dose.
- SAEs will be collected and reported as per PTCTC standard protocols.
- All serious and unexpected toxicities (Grade 3-4) **possibly, probably or definitely related** to NK infusions will be reported to the IRB and FDA within 15 days of occurrence of event

7.4 Adverse Event Reporting

Reporting of patient serious adverse events (SAEs) will be according to standard practices and requirements. Unexpected, Grades 3-5 AEs must be reported within 3 business days of knowledge of the event. Due to the expanded NK cells being regulated under an FDA IND application, all AEs (grades 1-5) up to at least **28 days** after the last study intervention (in other words, 28 days after the last NK cell infusion) will be reported. Other SAEs will be tracked periodically as defined in the EXCEL Study Manual of Procedures, staged according to NCI CTCAE, version 5.0. The DSMC will receive summary reports of all adverse experiences on at least an annual basis.

7.5 Reporting to the IRB

Local Site Principal Investigators will submit reports of unexpected SAEs and other unanticipated problems to local IRBs in an expedited fashion as required per institutional IRB policy. These events will be reviewed by the institutional IRBs.

7.5.1 Reporting Time Frames

Reports must be submitted to the PTCTC Study Coordinating Center in the following timeframes:

- Unexpected SAEs resulting in death and unexpected, Grade 3-5 AEs must be reported to the PTCTC Study Coordinating Center **within 3 business days** of the local Site Principal Investigator's knowledge of the event.
- All other SAEs and other unanticipated problems meeting the expedited reporting criteria must be reported to the PTCTC Study Coordinating Center **within seven business days** of the local Site Principal Investigator's knowledge of the event.
- All SAEs and Unanticipated Problems will be forwarded from the PTCTC Study Coordinating Center to the Sponsor-Investigator for review **within 3 business days** of receipt. These will be reviewed and discussed with the clinical sites within seven days of the report for any SAE or Unanticipated Problem.
- Upon receipt of the Protocol Chairs' assessment the Sponsor-Investigator is responsible for determining whether the SAE is related to the study and whether the SAE is unexpected. The Sponsor-Investigator will notify the appropriate regulatory groups based on the applicable regulations.

7.6 Pregnancy Reporting

Each pregnancy must be reported immediately (within 3 business days of identification) by email to the PTCTC Study Coordinating Center. All pregnancies will be reported to the CHLA IRB. Subjects who become pregnant after Day 0 will be followed to term, and the following information will be gathered for outcome, date of delivery, health status of the mother and child including the child's gender, height and weight. Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the treatment may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

7.7 IND Annual Report to the FDA

The site PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the IND sponsor-investigator. Each annual report will summarize study activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. The IND Sponsor-Investigator's representative will notify the site PI of the due date with sufficient time for the site PI to assemble the required information.

7.8 Final Report

A report will be submitted to the FDA in accordance with ICH E3 Guideline "Structure and Content of Clinical Study Reports".

8.0 CLINICAL TRIAL OVERSIGHT AND MONITORING

This protocol will be conducted in accordance with the standards of practice of the PTCTC and each study site. This protocol will be monitored in accordance with current Data Safety Monitoring Committee (DSMC) charter for investigator-initiated studies performed in the PTCTC network. The conduct of this clinical trial will be evaluated in accordance with PTCTC policies.

8.1 Safety monitoring

Safety monitoring will be conducted throughout the study; therefore, safety concerns will be identified by continuous review of the data by the Protocol Chair, clinic staff, and Data Safety Monitoring Committee (DSMC).

8.2 Data Safety Monitoring Committee (DSMC)

This study will be centrally reviewed and followed by the Data Safety and Monitoring Committee (DSMC) of the PTCTC.

The DSMC is a standing committee, composed of a chair, patient advocate, biostatistician, nurse representative and two BMT physicians with procedures and processes as defined in the DSMC Charter. The DSMC will review the study protocol prior to study activation and IRB review, and will continue to review the study on a regular basis according to the committee rules.

The DSMC will meet at regular intervals to review all adverse events and deaths and determine whether any patient safety problems necessitate protocol modifications or discontinuation of the trial. The DSMC

will also meet on an *ad hoc* basis if unexpected safety events occur that may necessitate study suspension or closure. The DSMC will discontinue the review of outcomes when this protocol is closed to accrual. Before each regularly scheduled DSMC meeting, the PTCTC Study Coordinating Center will submit a report including tabular summaries of all SAEs and deaths on study to date. The report will also include a brief summary of each previously unreported SAE and death, including an assessment of whether the event was unexpected or related to the study.

If the DSMC recommends protocol or informed consent changes during the study, the recommendations will be reviewed by the Protocol Chairs and incorporated into the protocol as deemed appropriate. The protocol with incorporated changes will be distributed to the participating study sites after approval by the Central IRB. It is the responsibility of each site PI to forward the distributed communications from the DSMC to their local IRB and provide updated approval information to the PTCTC Study Coordinating Center.

8.3 Study Monitoring

The site PI will permit study-related monitoring visits by representatives of the sponsor or designees, and regulatory inspections(s) (e.g., FDA) to ensure proper conduct of the study and compliance with all FDA safety reporting requirements. Access will be provided to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. The monitor will evaluate study processes and documentation based on FDA regulations and the International Council for Harmonization (ICH), E6: Good Clinical Practice guidelines (GCP) and 21CFR 312.

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9.0 LIST OF APPENDICES

10.1 Appendix A High Risk AML Mutations

10.2 Appendix B Busulfan Conversion Chart

10.3 Appendix C Acute GVHD Grading and Staging

10.4 Appendix D NIH Scoring for Chronic GVHD

10.5 Appendix E Comorbidity Index

9.1 Appendix A: List of High-Risk AML Mutations*

1. MECOM (3q26.2)
2. inv(3)(q21q26.2)
3. t(3;3)(q21;q26.2)
4. t(3;21)(q26.2;q22)
5. t(3;5)(q25;q34)(NPM1-MLF1)
6. t(6;9)(p23;q34.1)(DEK-NUP214)
7. Monosomy 7
8. Monosomy 5/5q-[EGR1(5q31) deleted]
9. KMT2A(MLL) (11q23.3)
10. t(4;11)(q21;q23)
11. t(6;11)(q27;q23)
12. t(10;11)(p11.2;q23)
13. t(10;11)(p12;q23)
14. t(11;19)(q23;p13.3)
15. NUP98 (11p15.5)
16. t(7;11)(p15;p15)
17. t(5;11)(q35.3;p15)(cryptic)
18. t(X;11)(q28;p15)/NUP98-HMGB3
19. t(2;11)(q31;p15/NUP98-HOXD13
20. 12p abnormalities (ETV6)
21. 12p loss or rearrangement
22. t(7;12)(q36;p13)
23. ETS fusion
24. FLT3 mutations, AR>0.1, excluding co-mutations with NPM1 or CEPBa
25. CBFA2T3-GLIS2
26. RAM phenotype
27. KAT6A(8p11.21) Fusion

*Other high-risk features not explicitly stated in this list can be considered after discussion and approval by the protocol chair/team

9.2 Appendix B: Busulfan Conversion Chart [96] (adapted from Seattle Cancer Care Alliance, Pharmacokinetics Laboratory. Version 1.1, updated 5.29.2019)

Total AUC (4-day regimen)		Q24H AUC (per dose)		Q6H AUC (per dose)		Css
mg×hour/L	µMolar×min	mg×hour/L	µMolar×min	mg×hour/L	µMolar×min	ng/mL
40.0	9744	10.0	2436	2.50	609	417
45.0	10962	11.3	2741	2.81	685	469
50.0	12180	12.5	3045	3.13	761	521
55.0	13398	13.8	3350	3.44	837	573
57.6	14032	14.4	3508	3.60	877	600
59.1	14400	14.8	3600	3.69	900	616
60.0	14616	15.0	3654	3.75	914	625
62.4	15200	15.6	3800	3.90	950	650
65.0	15834	16.3	3959	4.06	990	677
65.7	16000	16.4	4000	4.11	1000	684
67.2	16370	16.8	4093	4.20	1023	700
70.0	17052	17.5	4263	4.38	1066	729
72.0	17540	18.0	4385	4.50	1096	750
72.3	17600	18.1	4400	4.52	1100	753
73.9	18000	18.5	4500	4.62	1125	770
75.0	18270	18.8	4568	4.69	1142	781
76.8	18709	19.2	4677	4.80	1169	800
78.0	19001	19.5	4750	4.88	1188	813
78.8	19200	19.7	4800	4.93	1200	821
80.0	19488	20.0	4872	5.00	1218	833
81.6	19878	20.4	4970	5.10	1242	850
82.1	20000	20.5	5000	5.13	1250	855
85.0	20706	21.3	5177	5.31	1294	885
85.4	20800	21.4	5200	5.34	1300	889
86.4	21048	21.6	5262	5.40	1315	900
87.0	21200	21.8	5300	5.44	1325	907
90.0	21924	22.5	5481	5.63	1370	938
92.0	22400	23.0	5600	5.75	1400	958
95.0	23143	23.8	5786	5.94	1446	990
96.0	23386	24.0	5847	6.00	1462	1000
98.5	24000	24.6	6000	6.16	1500	1026
100.0	24361	25.0	6090	6.25	1523	1042
101.0	24604	25.3	6151	6.31	1538	1052

9.3 Appendix C: Acute GVHD Grading and Staging

aGVHD STAGING

STAGE	Skin	Upper GI	Lower GI	Liver
0	No active (erythematous) GVHD rash	No or intermittent nausea	<500 mL/day or <3 episodes/day for adult <10 mL/kg/day or <4 episodes/day for child	Bilirubin <2 mg/dL
1	Maculopapular rash <25% BSA	Persistent nausea, vomiting, or anorexia	500-999 mL/day or 3-4 episodes/day for adult 10-19.9 mL/kg/day or 4-6 episodes/day for child	Bilirubin 2-3 mg/dL
2	Maculopapular rash 25-50% BSA		1000-1500 mL/day or 5-7 episodes/day for adult 20-30 mL/kg/day for child	Bilirubin 3.1 - 6 mg/dL
3	Maculopapular rash >50% BSA		>1500 mL/day or >7 episodes/day for adult >30 mL/kg/day or >10 episodes/day for child	Bilirubin 6.1 - 15 mg/dL
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation >5% BSA		Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)	Bilirubin > 15 mg/dL

*For Children with BSA < 1.5 m^2, diarrhea volume should be recorded using mL/m^2 scale.

For skin GVHD:
Use “Rule of Nines” or burn chart to determine extent of rash.

For liver GVHD:
Range of bilirubin given as total bilirubin. Downgrade one stage if an additional cause of hyperbilirubinemia is documented.

For gut GVHD:
Downgrade one stage if an additional cause of diarrhea is documented. Stage 1 is persistent nausea, vomiting and anorexia in the absence of other known cause unless histology is negative.

aGVHD GRADING

GRADE	Skin	Liver	Gut
0	None	None	None
I	Stage 1-2	None	None
II	Stage 3 and/or	Stage 1 and/or	Stage 1
III	None or Stage 3 and/or	Stage 2-3 and/or	Stage 2-3
IV	Stage 4 or	Stage 4	NA

Criteria for grading given as minimum degree of organ involvement required to confer that grade.

9.4 Appendix D: NIH Scoring for Chronic GVHD

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LUNGS[†] FEV1 ☐ DLCO ☐	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
	☐ FEV1 > 80% **OR** LFS=2	☐ FEV1 60-79% **OR** LFS 3-5	☐ FEV1 40-59% **OR** LFS 6-9	☐ FEV1 ≤39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable (none – 0,mild -1, moderate -2, severe – 3)

Esophageal stricture or web___ Pericardial Effusion___ Pleural Effusion(s)___

Ascites (serositis)___ Nephrotic syndrome___ Peripheral Neuropathy___

M yasthenia Gravis___ Cardiomyopathy___ Eosinophilia > 500µl___

Polymyositis___ Cardiac conduction defects___ Coronary artery involvement___

Platelets <100,000/µl ___ Progressive onset___

OTHERS: Specify:______________________________

9.5 Appendix E: Comorbidity Index

Directions for completing the HCT-CI worksheet:

1) If the recipient has a documented history of any of the conditions listed in the "definition/compartments" column, check the corresponding "yes" box.
2) If any box within a category is checked "yes", then the number in the "score" column should be counted.
3) The total of all the boxes with a score represents the final score of the index.

Co-morbidity	Definition/compartments	Yes	Score
1. Arrhythmia	-Atrial fibrillation* -Atrial flutter* -Sick sinus syndrome* -Ventricular arrhythmia*	☐ ☐ ☐ ☐	1
2. Cardiovascular	-Coronary artery disease* -Congestive heart failure* -Myocardial infarction* -Ejection fraction ≤50%§	☐ ☐ ☐ ☐	1
3. Inflammatory bowel disease	-Crohn's disease* -Ulcerative colitis*	☐ ☐	1
4. Diabetes	-Treated with insulin or oral hypoglycemic drugs§	→	1
5. Cerebro-vascular	-Transient ischemic attacks* -Cerebro-vascular ischemic or hemorrhagic stroke*	☐ ☐	1
6. Depression/anxiety	-Requiring psychological consultation and/or specific treatments§	→	1
7. Hepatic - mild	-Chronic hepatitis§ -Bilirubin >ULN- 1.5 X ULN§ -AST/ALT >ULN- 2.5 X ULN§	☐ ☐ ☐	1
8. Obesity	-Body mass index >35 (adults)§ -Body mass index-for-age ≥95% percentile (children)§	☐ ☐	1
9. Infection	-Requiring anti-microbial treatment before, during, and after the start of conditioning§	→	1
10. Rheumatologic	-Requiring Treatment*	→	2
11. Peptic ulcer	-Confirmed by endoscopy and requiring treatment*	→	2
12. Renal	-Serum creatinine >2mg/dl (or >177μmol/L)§ -On dialysis§ -Prior renal transplantation*	☐ ☐ ☐	2
13. Pulmonary - Moderate	-DLco corrected for hemoglobin 66-80% of predicted§ -FEV1 66-80% of predicted§ -Dyspnea on slight activity§	☐ ☐ ☐	2
14. Pulmonary - Severe	-DLco corrected for hemoglobin ≤ 65% of predicted§ -FEV1 ≤ 65% of predicted§ -Dyspnea at rest or requiring oxygen therapy§	☐ ☐ ☐	3
15. Heart valve disease	-Except asymptomatic mitral valve prolapse§	→	3
16. Prior solid malignancy	-Treated with surgery, chemotherapy, and/or radiotherapy, excluding non-melanoma skin cancer*	→	3
17.Hepatic - moderate/severe	-Liver cirrhosis§ -Bilirubin > 1.5 X ULN§ -AST/ALT > 2.5 X ULN§	☐ ☐ ☐	3
		Total Score	

*Diagnosed at any time in the patient's past history
§Detected at the time of pretransplant assessment - ULN indicates upper limit of normal; DLco, diffusion capacity of carbon monoxide; FEV1, forced expiratory volume in one second; AST, aspirate aminotransferase; and ALT, alanine aminotransferase