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A Phase 1/2 Study of the Safety and Efficacy of Chemotherapy Combined with Adoptive Transfer of HLA-Haploidentical Donor Lymphocyte Infusion in Older Patients with High-Risk Acute Myeloid Leukemia and Myelodysplastic Syndrome

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1.0 INTRODUCTION

The outcome of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in patients ≥55 years still remains unsatisfactory, with a low complete remission (CR) rate and poor overall survival (OS) due to a variety of reasons including increased incidence of poor-risk cytogenetics, increased activation of RAS, Src, and TNF pathways and intrinsic resistance of leukemic blasts to therapeutic agents. The 30-day induction mortality is also high and ranges from 10-50% and this is due to prolonged pancytopenia, febrile neutropenia and other systemic side effects that burden the already aging patient with comorbidities. [1-3]. Allogeneic stem cell transplantation (alloSCT) has curative potential; however, it is associated with severe graft-versus-host disease (GVHD), infections, and other treatment related morbidities or mortality for a high proportion of elderly patients [4, 5]. Thus, recent work has centered on less toxic ways to manipulate donor cells to achieve antitumor benefits.

A pilot study performed in China in elderly patients with AML treated with conventional chemotherapy followed by infusion of human leukocyte antigen (HLA) mismatched G-CSF mobilized peripheral blood stem cell infusion (G-PBSC) reported higher CR and disease-free-survival (DFS) rates, faster neutrophil and platelet recovery, and no GVHD or increase in treatment-related mortality [6]. Interestingly, although no patients exhibited full or mixed chimerism, donor microchimerism was monitored and observed in all four women who had male donors (sons or brothers). This may be due to long-term maternal-fetal microchimerism, and studies of HLA-haploidentical allo-SCT have shown improved outcomes after transplantation from noninherited maternal antigen (NIMA) mismatched donors [7]. Improved responses after HLA-mismatched G-PBSC infusion may also be due to natural killer (NK) cells: infusion of haploidentical NK cells from killer immunoglobulin-like receptor (KIR) ligand mismatched donors have shown alloreactivity against leukemia [8, 9]. Because induction and consolidation chemotherapy followed by infusion of HLA-haploidentical cells, or donor lymphocyte infusion (DLI), is a new procedure, our first priority is to replicate the above study as a Phase I trial to assess safety.

Our primary hypothesis is that chemotherapy followed by infusion of HLA-haploidentical DLI from KIR-ligand mismatched donors is a safe procedure that will not cause GVHD or increased treatment-related mortality. We further believe that this will improve outcomes of elderly patients with high-risk AML or MDS compared to chemotherapy alone, and that that this benefit will be even greater in donor-recipient pairs that share maternal-fetal microchimerism or NIMA-mismatch. A large part of this trial will include immune function assays as well as assessments of efficacy, toxicity, and GVHD. Because this therapy may be a tolerable alternative to alloHSCT for elderly patients, we will validate functional measurements (e.g. Comprehensive Geriatric Assessment (CGA)) with biologic correlates (cytokine and genomic profiles) and clinical outcomes.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

2.1.1 Evaluate the safety of HLA-haploidentical DLI combined with conventional induction and consolidation chemotherapy in terms of unacceptable toxicity as defined in section 7.0.

2.1.2 Evaluate the efficacy of HLA-haploidentical DLI combined with conventional induction and consolidation chemotherapy in terms of one-year DFS.

2.1.3 Validate the use of the CGA in elderly AML and MDS patients undergoing cellular therapy

2.2 Secondary Objectives

2.2.1: Evaluate incidence and severity of acute GVHD.

2.2.2: Evaluate incidence and severity of other adverse events.

2.2.3: Evaluate efficacy with respect to CR and OS.

2.2.4: Evaluate immune recovery.

2.2.5: Evaluate hematopoietic recovery.

3.0 BACKGROUND

3.1 High-Risk AML and MDS in the Elderly

Elderly AML and MDS patients have long been known to have much worse rates of CR, DFS, and OS compared to their younger counterparts [1, 3]. The CR rates in elderly AML patients treated with standard induction chemotherapy (7+3, i.e. 7 days of cytarabine and 3 days of anthracycline) is ~30-65%, with more than half of these patients relapsing within six months [1, 10], and survival in high-risk MDS is only 26 months vs. 141 months for very low risk[11]. In part, this is because elderly patients are less likely to have favorable cytogenetics: an analysis of 1065 elderly patients with AML found 7% to have favorable cytogenetics, 73% intermediate, and 20% poor, with complete remission rates of 72%, 53-63%, and 26% respectively, and five-year overall survival rates of 34%, 10-15%, and 2% respectively [12]. However, even with favorable cytogenetics, patients older than 65 have poor outcomes with <10% two-year overall survival [1].

3.2 Stem Cell Transplant for Elderly High-Risk AML and MDS

Nonmyeloablative or reduced intensity conditioning (RIC) allogeneic stem cell transplantation (alloSCT) does have curative potential for elderly patients with AML and MDS; however, it is associated with severe graft-versus-host disease (GVHD), infections, and organ toxicity that may lead to unacceptably high rates of morbidity and mortality [4, 5, 13]. Further, the lack of an available human leukocyte antigen (HLA)–matched donor remains a major obstacle for many patients. Haploidentical transplantation may provide a larger pool of available donors, but it is limited by significant morbidity and mortality (e.g., GVHD and infections) [14]. DLI can also mediate strong graft-versus-leukemia (GVL) effects but may be associated with severe GVHD and delayed neutrophil recovery after alloSCT [15, 16].

3.3 Graft versus Leukemia (GVL) Effect

Most patients with hematologic malignancies are not cured with standard chemotherapy. Outcomes are improved by alloSCT, which can induce an immunologic effect against the cancer. In patients with leukemia, this has been termed a graft versus leukemia effect. Evidence for this includes:

1. temporal relationship between graft versus host disease and hematologic remission [17]
2. reduced incidence of leukemic relapse after alloSCT compared to syngeneic SCT [18]
3. reduced incidence in leukemic relapse in alloSCT recipients who do develop GVHD compared to those who do not [19]

Multiple laboratory studies have been published in support of this clinical data as well. Tumor specific antigens, histocompatibility antigens, and killer immunoglobulin receptors (KIR) may play a role through direct cell contact, activating NK cells, or indirect production of cytotoxic cytokines (interferon, TNF-alpha, IL-2, IL12).

3.4 Donor Leukocyte Infusions as a way to Induce GVL Effect

Recent work with patients who have relapsed or have persistent disease post-alloSCT has centered on using donor leukocyte infusions to stimulate more graft versus tumor effect and re-attain remission [20, 21]. Reports are growing of clinically significant results in various types of malignant and non-malignant processes, and it is now the standard of care to proceed with donor leukocyte infusions in patients who have relapsed disease post allogeneic transplantation [22].

For non-selected DLIs, donors are asked to provide peripheral blood progenitor cells in the same manner as for transplantation; these are then transfused through a peripheral intravenous line into the recipient. Typically, somewhere between 10^5 to 10^6 CD3+ T cells/kg recipient weight are infused over 1 to 2 days every 4-12 weeks, though there is no set standard. Although higher doses are associated with increased anti-tumor immunity, they are also associated with increased GVHD. The time between infusions allows for observation and management of pancytopenia, the anticipated side effect if significant graft versus tumor effects is encountered along with suppressive effects on the patient's innate hematopoiesis. Immunosuppressive agents are continued during leukocyte infusions; however, if clinically significant graft versus host disease does occur, treatment may be initiated to control the degree of response at that time.

3.5 NK Alloreactivity

Donor-versus-recipient NK alloreactivity has emerged as a crucial factor for the outcome of haploidentical SCT [8]. Alloreactivity is mediated by KIR-ligand mismatch between donor and recipient [23]. Ruggeri et al showed a low relapse risk for patients with acute myeloid leukemia transplanted from NK-alloreactive donors [9]. Further, adoptive transfer of alloreactive haploidentical NK cells has been shown to be safe and effective in treatment

of AML [24, 25]. NK-enriched DLI may also improve outcomes and immune recovery [26].

3.6 NIMA and Maternal-Fetal Microchimerism

Outcomes after HLA-haploidentical allo-SCT are better when using the patient's mother as the donor compared to the father [7, 27, 28]. This is thought to be secondary to in-utero exposure of NIMA, leading to the establishment of long-term maternal-fetal microchimerism, which can be found in 40% of adults [29] and lead to both tolerogenic as well as sensitizing effects [30, 31]. One study by Stern et al found the five-year event-free survival rate was 50% in patients who had transplants from their mother versus 11% for those who had transplants from their father, in large part due to reduced incidence of relapse, thought secondary to improved graft-versus-leukemia[27]. Maternal-fetal microchimerism may also mediate a graft-versus-leukemia effect in cord blood transplantation [32]. Thus, adoptive transfer of cells from mother to child or child to mother may improve outcomes.

3.7 Infusion of HLA-mismatched peripheral blood stem cells in elderly AML

A recent Chinese study reported that mice infused with a high dose of G-CSF–mobilized allogeneic spleen cells (3-12 $x10^7$) after cytarabine chemotherapy exhibited rapid hematopoietic recovery and persistent microchimerism without GVHD despite absence of GVHD prophylaxis and immunosuppressive treatment [6]. On the basis of these observations, they designed a clinical control study to investigate the effects of G-PBSC combined with conventional induction and intensive postremission chemotherapy on outcomes of AML in elderly patients. Fifty-eight AML patients aged 60-88 years were randomly assigned to receive induction chemotherapy with cytarabine and mitoxantrone (control group; n=28) or same chemotherapy plus HLA-mismatched G-PBSCs (G-PBSC group; n=30) (median CD3+ cell dose: 0.9 x 10E8 CD3+ cells/kg recipient body weight). Patients who achieved CR received another 2 cycles of postremission therapy with intermediate-dose cytarabine plus G-PBSCs. The complete remission rate was significantly higher in the G-PBSC group than in the control group (80.0% vs. 42.8%; *P*= .006). The median recovery times of neutrophils and platelets in the G-PBSC group were 11 days and 14.5 days, respectively, and 16 days and 20 days in the control group. The 2 year probability of disease-free survival was significantly higher in the G-PBSC group than in the control group (38.9% vs. 10.0%; *P* =.01). No graft-versus-host disease was observed in any patient.

Given that none of these patients experience engraftment, yet there was a significant improvement in disease free survival, we hypothesize that these results were due to infused donor lymphocytes rather than CD34+ stem cells. As a result, G-PBSC may not be necessary and HLA-haploidentical DLI would suffice.

3.8 Geriatric assessment

To better understand the mechanisms that contribute to the poor prognosis of

elderly AML patients, our laboratory has demonstrated that differences in the underlying biology (higher probability of Src, TNF, and RAS pathway activation in elderly AML patients as compared to their younger counterparts) contribute to poor survival[33]. We will build on this knowledge by using the CGA to improve our clinical assessment of functional status in elderly AML and MDS patients to predict responses to and toxicity from induction therapy as well as mortality and correlate these results with biologic markers of frailty using cytokine and genomic profiles.

4.0 RATIONALE

Elderly patients with high-risk AML and MDS respond very poorly to conventional induction regimens and have high relapse rates. AlloSCT is one way of decreasing relapse but at the cost of toxicity and GVHD. Infusion of HLA-haploidentical stem cells may provide an alternate means of inducing a graft-versus-leukemia effect with less toxicity. This may be mediated by NK-alloreactivity and potentiated by maternal-fetal microchimerism. Our trial will attempt to confirm the safety and efficacy of the Chinese study in an attempt to improve 2-year survival in elderly high-risk AML and MDS patients, with minimal toxicity and GVHD, with a focus on immunological correlates and function in an attempt to further understand the mechanism of this phenomenon.

We have selected the dose of 1 x 10E8 CD3+ cells/kg recipient body weight based on the success of this dose in the study by Guo et. al.[6] and the use of 1 x 10E8 – 2 x 10E8 CD3+ cells/kg recipient body weight by Colvin et. al. in a similar study[34]. In both the Guo and Colvin studies, GVHD was not seen at this dose.

5.0 ELIGIBILITY CRITERIA

Laboratory, bone marrow biopsy, and radiographic evaluation to determine eligibility should be done within 4 weeks of registration. Baseline staging studies not needed for eligibility but used to assess disease prior to transplantation may be performed up to 8 weeks before registration.

5.1 Patient Eligibility

1. Subjects must have their diagnosis of high-risk AML or high-risk MDS confirmed by pathologic review of bone marrow biopsy according to WHO guidelines [35] (see below for definitions of high-risk)
    - We will define patients as high risk AML and thus eligible if they meet one or more of the following criteria:
        a. Secondary AML (from underlying MDS or therapy related)
        b. Presence of complex cytogenetic abnormalities ($\geq$ 3 cytogenetic abnormalities), all monosomies, del 5q, del 7q, inv3, t(3;3), t(6;9), t(9;22), abn 11q23 (excluding t(9;11))[36]
        c. FLT3-ITD mutation positive
        d. Age $\geq$65 years given poor outcomes even with favorable

cytogenetics[1]

- We will define subjects as high risk MDS and thus eligible if they have a MD Anderson Comprehensive Cancer MDS Risk Score ≥9[3]

2. Subjects must have ECOG Performance status of 0,1,or 2; if ECOG 2, they must also have a Charlson comorbidity index of ≤5.[37]
3. Subjects must be ≥ 55 years of age
4. Subjects should have a potential 3-5/6 HLA-matched related haploidentical donor that will be evaluated for eligibility to provide DLI.
5. Patient should be able to provide informed consent
6. Subjects must have a MUGA and /or ECHO or cardiac MR. The required minimum standards include MUGA or ECHO or cardiac MR showing an EF of 40%. Those with an EF 40-49% must also have a cardiologist consult and assist with management.
7. PFTs with DLCO are conditional for subjects at the discretion of the physician. The required minimum standards for those who have PFTs include DLCO of 40%. Those with DLCO of 40-49% must have a pulmonologist consult and assist with management.
8. Subjects of all genders and races are eligible

5.2 Exclusion criteria

1. Pregnant or lactating women.
2. Patients with other major medical or psychiatric illnesses which the treating physician feels could seriously compromise tolerance to this protocol
3. Patients with known active CNS disease
4. Patients with acute promyelocytic leukemia (FAB M3)

5.3 Donor selection

1. Adult donors must be must be a HLA 3-5/6 related haploidentical match with the patient and must be capable of providing informed consent
2. Donors must not have any medical condition which would make apheresis more than a minimal risk, and should have the following:
   a. Family members will be considered for donation if they do not have a history of known cardiac problem and do not have abnormal cardiac findings by physical examination. Those with a history of cardiac problems or abnormal cardiac findings by physical examination should undergo a stress evaluation or be evaluated by a cardiologist and deemed eligible to donate
   b. Bilirubin and hepatic transaminases $\leq$ 2.5 x ULN,
   c. Adequate hematologic parameters including a hematocrit > 35% for males and 33% for females, white blood cell count of ≥ 3,000, and platelets ≥80,000.
   d. FACT labs must be drawn within 7 days of collection and final test results available prior to infusion into the patient (copy of labs included in appendices). In the case of multiple donations from the donor, the FACT labs must be redrawn within 7 days of each initiation of apheresis (positive serologies are not repeated as they remain positive for lifetime but all other donor labs are performed as per the donor appendix sheet).

3. Females of childbearing potential should have a negative serum beta-HCG test within 1 week of beginning growth factor of choice

5.3.1 Donor selection algorithm

Preference is first given to:

a. the best matched family member who meets other donor criteria, followed by
b. age (younger donor preferred)
c. KIR reactivity according to the following rules:
   i. If a recipient is missing HLA-C group2 ligand, a preferable donor has an inhibitory KIR to HLA-C group2 ligand (KIR2DL1)
   ii. If a recipient is missing HLA-A3/A11 ligand, a preferable donor has an inhibitory KIR to HLA-A3/A11 ligand (KIR3DL2)
   iii. If a recipient is missing HLA-Bw4 ligand, a preferable donor has an inhibitory KIR to HLA-Bw4 ligand (KIR3DL1)
   iv. A preferable donor has more than one inhibitory KIR (2DL1/2/3, 3DL1/2) without corresponding ligand in a recipient, followed by
d. CMV negativity
e. NIMA mismatch over NIPA mismatch

6.0 TREATMENT PLAN

6.1 Prior to starting therapy

Prior to enrollment, potential subjects will have a complete history and physical examination performed as well as the following required lab tests: CBC with differential (auto or manual), creatinine, AST, ALT, total bilirubin,and bone marrow biopsy with cytogenetics and molecular mutation studies. These should all be completed within four weeks prior to enrollment. Cardiopulmonary testing as per section 5.1 must be completed within eight weeks. Other recommended tests (not required but helpful for final analysis) include: CMV immune screen, LDH, uric acid, alkaline phosphatase, sodium, potassium, chloride, bicarbonate, glucose, total protein, albumin, calcium, magnesium, phosphorous.

If a potential subject is awaiting the above required lab tests to assess suitability for enrollment (e.g. normal cytogenetics and FLT-3 mutation testing), and if WBC is elevated and/or rising, hydrea or leukapheresis may be initiated at the discretion of the treating physician.

6.2 Donor priming and apheresis

Exam and laboratory analyses will follow current Foundation for the Accreditation of Cellular Therapy (FACT) standard of care accreditation requirements for cellular therapy practices.

6.2.1 Mobilization

Recommended mobilization includes G-CSF 8 mcg/kg subcutaneously twice daily for 4 days prior to stem cell collection and continuing until pheresis is

completed. Dose should be rounded to nearest vial size. Alternative mobilization strategies may be employed at the investigator's discretion.

6.2.2 Collection of cells

General guidelines (though may be altered for program standards and evolving stand of care without being a violation): The goal is to collect 3 x 10^8 CD3+ cells/kg recipient body weight. If, after a maximum of 2 leukaphereses, the target collection is not reached, the treating physician may decide to proceed with a third collection after re-discussion with the patient and donor. If the goal dose is not reached, infusion will still take place if a minimum of 2x x 10^8 CD3+ cells are collected, and recovery will be monitored as the patient will normally have autologous recovery.. The infusion schedule will be modified as below (6.3.4). Leukapheresed cells will be processed and frozen in the cryopreservation lab according to standard procedures, unless they are to be immediately infused into the patient after processing (e.g. freshly collected cells to be given following induction).

The donor lymphocyte infusion meets criteria to be regulated solely as HCT/P instead of a drug/biologic. There is no manipulation of donor cells and the donor is a first or second degree relative.

6.2.3 Storage and processing

The stem cells are processed in the Duke University Medical Center Stem Cell Laboratory and infused to the donor following the standard laboratory procedures for allogeneic stem cell infusions. Freshly collected cells will be used in the first course of treatment and donor cells will be divided into aliquots and cryopreserved in liquid nitrogen for subsequent infusions. Each infusion of the cells will be performed 1 day following each chemotherapy regimen and that day will be counted as 'day 0'. The target dose of CD3+ cells is 1x10^8 cells/kg (the collection goal of 3x10^8 CD3+ cells/kg is to obtain 1 aliquot of 1x10^8 CD3+ cell/kg for each of induction and consolidation2, with 1x10^8 CD3+ cell/kg saved in case of need for re-induction). If only 2-3x10^8 CD3+ cell/kg is collected, then cells will only be given with IND1 and CON2 (IND2 may still be done, but cells will not be given). We will also monitor the total number of mononuclear, CD34+, CD3-CD16+CD56+ (NK) cells, CD3+CD16+CD56+ (NKT) cells, and gamma-delta T-cells infused, but the infusion dose will be calculated around CD3+ population.

6.3 Treatment Plan

6.3.1 Induction therapy

Eligible subjects will receive induction chemotherapy with idarubicin (12 mg/m^2 intravenously for 3 days) and cytarabine (100 mg/m^2 intravenously for 7 days) starting Day 1 and ending Day 7 as described in Figure 1. Patients 80 years or older may receive 2 and 5 days of idarubicin and cytarabine respectively starting Day 1 and ending Day 5 (optional, at physician discretion). HLA-mismatched DLI will be administered Day 9

(DLI will occur on Day 7 for 5+2 regimen), approximately 24-48 hours following completion of chemotherapy at a dose of 1x10^8 CD3+ cells; however, due to logistics of planning infusions with staffing, donor availability, weekends/holidays, etc., it may be necessary to postpone cell infusion up to 96 hours, which will be allowed without being considered a protocol violation.

Subjects who experience no remission or partial remission will receive a second course of the identical induction chemotherapy (IND2). IND2 should include 7+3+DLI as per IND1, but if cells are not available as per 6.2.3, chemotherapy alone can be given without being considered a protocol violation.

Given the time constraints presented by the need to start induction chemotherapy as soon as possible, in some cases, it may not be logistically possible to administer cells with induction (e.g., obtaining insurance approval for clinical trial/DLI may take a while, and approval may not be back in time for a patient to receive cells). In these cases, patients would just receive standard induction chemotherapy and cells would be administered after CON1 in addition to CON2 (see below). Timing of correlative laboratory studies will be adjusted to match cell infusion. Guo et al. have reported improved outcomes with high cell doses (1x 10^8 CD3+ cells/kg) administered after consolidation, even in patients who receive standard induction chemotherapy without cells.[1]

[1] Guo M, Hu KX, Liu GX, Yu CL, Qiao JH, Sun QY, Qiao JX, Dong Z, Sun WJ, Sun XD, Zuo HL, Man QH, Liu ZQ, Liu TQ, Zhao HX, Huang YJ, Wei L, Liu B, Wang J, Shen XL, Ai HS. HLA-mismatched stem-cell microtransplantation as postremission therapy for acute myeloid leukemia: long-term follow-up. J Clin Oncol. 2012 Nov 20;30(33):4084-90. doi: 10.1200/JCO.2012.42.0281. Epub 2012 Oct 8.

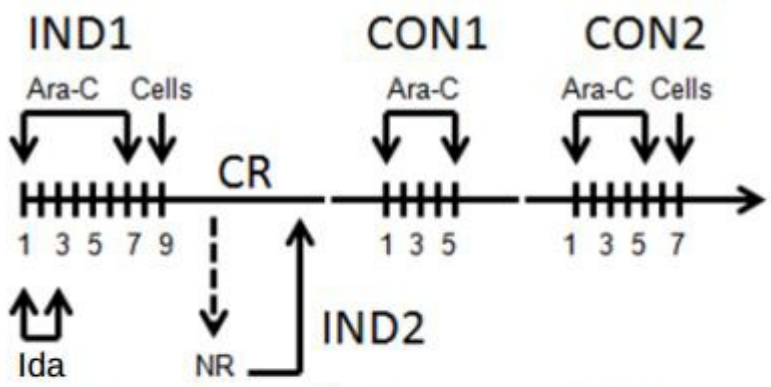

Figure 1: Chemotherapy followed by adoptive transfer of HLA-haploidentical donor lymphocytes. Patients will receive induction chemotherapy followed by cell infusion, and then 2 rounds of consolidation chemotherapy followed by cell infusion. If patients do not achieve complete remission after the first round of induction, they may undergo a second round. Ara-C, cytarabine; Cells, adoptive transfer of HLA-haploidentical donor lymphocytes; CON1, first consolidation; CON2, second consolidation; CR, complete remission; Ida, idarubicin; IND1, first induction chemotherapy; IND2, second induction chemotherapy; NR, no response

6.3.2 Postremission treatment

Subjects who achieve a CR will receive 2 further courses of cytarabine postremission chemotherapy (consolidation) at 1.0 g/m^2 for 6 dosages followed by HLA-mismatched DLI after the second consolidation. Cytarabine will be given twice daily at 12 hour intervals on days 1, 3, and 5; HLA-mismatched DLI will be administered the day following the completion of the second consolidation (day 7) at a dose of 1x10^8 CD3+ cells, although as above, a 24-96 hour interval between completion of chemotherapy and infusion of cells will be allowed for logistical reasons without being considered a protocol violation.

No cells will be given after the first consolidation, as chemotherapy given with the second consolidation will likely kill any cells infused after the first consolidation (i.e. infusion of cells after the first consolidation would be costly and low-yield) (Note that while this argument could also be applied to cells infused after induction, Guo et al. showed an improvement in the CR rate from 40% to 80% with the use of cells with induction, which is why we plan to use cells after induction in this trial).

The timing of consolidation typically takes place after count recovery, around 6-12 weeks after induction, following standard protocols and timing for cytarabine consolidation.

6.3.3 Dose Adjustments

Patients with Hepatic Impairment Dosing: Dosage will be modified depending on clinical response and degree of hepatic impairment, but no quantitative recommendations are available.

Subjects with Renal Impairment Dosing:
Our program follows the following guidelines: Estimate a creatinine clearance using the formula for the estimated CrCl in ml/min:

(140- age)/serum Cr x (ideal body wt/72).
In addition, for females this value is multiplied by .85

For subjects with a CrCl > 60 ml/min: No dosage adjustment.
CrCl <= 60 ml/min: There is an increased risk of cerebellar and cerebral toxicity with high dose regimens. The recommended dosage adjustment will be as follows: For a serum creatinine of 1.5—1.9 mg/dl or increase of serum creatinine from baseline of 0.5—1.2 mg/dl during treatment, the dose of cytarabine will remain 100 mg/m^2 during induction and be reduced to 500 mg/m^2 during postremission therapy. For a serum creatinine ≥ 2 mg/dl or if the change in baseline serum creatinine was > 1.2 mg/dl, we will reduce the dose of cytarabine to 50 mg/m^2 during induction and to 250 mg/m^2 during postremission therapy.

6.3.4 DLI

Allogeneic DLI will be collected from the donor as described in section 6.2.2. The allogeneic DLI will be infused as per current practice. Subjects will be given premedications including Benadryl 25 mg IV, and Acetaminophen 650 mg po prior to infusion. Hydrocortisone is used at the physician's discretion.

If needed, modifications to the infusion rate may be made as per standard practice.

6.3.5 Supportive Care

GVHD prophylaxis: GVHD prophylaxis will not be administered.

Growth factor use: A growth factor of choice may be initiated at the treating physician's discretion.

Antibiotic prophylaxis (may be altered to follow the treating physicians standard practice without affecting overall study)**:**

a. Voriconazole 200 mg PO BID or Posaconazole 200 mg PO TID beginning at day 9 (or Day 7 for subjects receiving 5+2 regimen) until CD4+ >200/uL or 1 year following therapy, whichever occurs first. Alternatives may be substituted at the physician's discretion.

b. Acyclovir 400 mg PO BID beginning day 9 (or Day 7 for subjects receiving 5+2 regimen) until ANC > 1000/uL or day +30 if a donor or recipient is only HSV seropositive; acyclovir 800mg PO BID will continue from day +30 through 1 year or until CD4 count is >200/uL if a donor or recipient is VZV seropositive.
c. Ciprofloxacin 500mg po BID should be initiated beginning on day 9 (or Day 7 for subjects receiving 5+2 regimen) and continue until CD4 count >200/uL or 1 year following therapy if tolerated orally. Alternative prophylactic antibiotics may be used at the physician's discretion.
d. CMV monitoring will occur at baseline. The treating physicians may use their own test of choice for this surveillance, though a PCR based assay is recommended. A positive test necessitates initiating therapy per local standards (low positive typically requires a repeat confirmation and a high positive immediate initiation of anti-CMV therapy with ganciclovir or foscarnet).

Blood products: All blood products (except DLI) will be irradiated following local standards to prevent possible GVHD. Hematocrit should be maintained at $\geq$ 25%, and platelets at > 10,000/mm^3 (subject to treating physician discretion). All blood products should be filtered prior to infusion

Other care: Agents designed to minimize other side effects of transplantation such as decreasing mucositis are allowed at the physician's discretion.

6.4 Subject Evaluation

6.4.1 Clinical Evaluation

Subjects will be evaluated daily during induction and recovery from therapy while admitted to the hospital. Evaluation will include history and physical exam and toxicity monitoring, including acute GVHD monitoring. They will return for post-remission therapy and typically be re-admitted to our service for further treatment. In selected cases, subjects may remain outpatient with very close care and follow up per the treating team's discretion. Upon completion of therapy, they will be seen at least every other week until 8 weeks, after which they will be seen at least every 3 months for 2 years.

6.4.2 Laboratory and other testing

(Correlative Studies for 00043247 v. 15.06.19 Lab Manual)

Evaluation for supportive care during therapy/recovery and after therapy will be as follows (Recommended but may change based on clinical status per treating physician):

(a) CBC will be obtained daily during induction until transfusions of platelets and red blood cells are not regularly required, then as clinically indicated. When the white count reaches greater than

500/uL, then add differential and follow to document when ANC > 1,500/uL. After consolidation, CBC and differential will be checked every 2 weeks or as clinically indicated.

(b) Serum chemistries and liver functioning tests at least weekly and as clinically indicated while during therapy/recovery; chemistries and liver function tests will also be checked at 8 weeks and then every 3 months for 2 years.

(c) Serum quantitative beta-HCG will be checked one week prior to induction in females.

(d) Biopsies of organs suspected to be involved with GVHD as clinically indicated (e.g., skin, liver, GI tract).

(e) Chest X-ray as clinically indicated.

(f) Bone marrow biopsy at Day 14 (per investigator discretion) as well as after count recovery (mandatory)

(g) HLA Antibodies at count recovery and 8 weeks after CON2.

(h) For patients who relapse or have refractory disease going off study, collect the following labs: peripheral blood for minimal residual disease testing, NK lysis assay, microchimerism, TCR sequencing, PD1/PDL1/PDL2 expression.

6.4.3 Detection of donor chimerism and microchimerism:

Hematopoietic donor chimerism will be assessed in all subjects with the use of standard cytogenetic and semiquantitative PCR-based analysis of short tandem repeats with 1% sensitivity. Microchimerism will be analyzed using real time PCR (qPCR) at a sensitivity of 1/100,000.

<u>These assays will be performed at count recovery prior to each new cycle of therapy, at 8 weeks after the last round of consolidation, and if still positive, every 3 months after completing therapy for up to two years. Additionally, in the case of mother-child pairs or sibling pairs that are mismatched in NIMA, this will be conducted before first infusion to assess for persistent maternal-fetal microchimerism.</u>

6.4.4 Evaluation of immune function

We will evaluation immune function based peripheral blood and bone marrow analysis using the following assays:

1. Cytokines will be measured, particularly levels of IL1, IL1beta, CRP, IL2, IL6, IL8, IL15, IL21, IFN-gamma, TNF-alpha, TGF-beta
2. Lymphocyte subsets will be analyzed using flow cytometric lymphocyte immunotyping with fluorochrome conjugated monoclonal antibodies to CD3, CD4, CD8, CD11c, CD16, CD19, CD25, CD28, CD45RA/RO, CD56, CD57, CD62L, CD107, CD123, TCR gamma-delta, FOXP3, HLA, and KIR
3. Quantification of recent thymic immigrants will be determined by the presence of TCR rearrangement excision circles (TREC), retrospectively performed by real-time quantitative-PCR of DNA collected from an isolated fraction of CD31 T cells and next

generation sequencing to analyze diversity in the TCR repertoires produced by the rearrangements of the variable region genes.

4. NK activity will be assessed by FACS (CD56+CD107+).

These assays will be done at count recovery prior to each new cycle of therapy, at 8 weeks after the last round of consolidation, and every 3 months after completing therapy for up to two years. Additional blood will not be drawn for this testing.

6.4.5 T cell receptor sequencing

T-cell recovery and repertoire diversity will also be analyzed using next-generation DNA sequencing of the complementarity determining region 3 (CDR3) of T-cell receptor (TCR) beta chains using the Illumina platform. The effectiveness of deep sequencing has been reported by van Heijst et al., who successfully used 5' RACE and deep sequencing to analyze TCR diversity after alloHSCT, demonstrating that this is strategy is feasible and that results are reproducible[38]. Intriguingly, in their study, van Heijst et al. were able to identify a clonal population of T cells associated with EBV relapse, suggesting that TCR deep sequencing may provide insight into ongoing anti-infectious and anti-tumor immunity. We have conducted our own pilot studies of TCR sequencing through our Immune Core, lead by our collaborator, H. Kim Lyerly, M.D., using frozen tumor biopsies from the Duke Breast SPORE tissue bank, demonstrating our ability to apply this technology as well evidence of oligoclonality and antigen-specific immune responses in cancer.

Working with Dr. Lyerly and other collaborators, we will use deep sequencing of TCR genes to analyze T cell recovery and TCR repertoire after chemotherapy and infusion of HLA-haploidentical donor lymphocytes. Starting with 5' RACE, which allows for the linear, unbiased and comprehensive amplification of all TCRs irrespective of the TCR beta variable (TRBV) or joining (TRBV) gene usage, or other primer-based methods (e.g. Adaptive), we will then use real-time PCR to add sequences that incorporate barcodes and Illumina flow cell-binding sequences while restricting amplification to the linear phase. After cluster generation by bridge PCR, 200bp paired-end sequencing reactions are then performed using MiSeq and HiSeq2000 sequencers. TRBV, TRBJ and TRBV-TRBJ usage frequencies are initially determined as well as repertoire diversity using Shannon entropy and Simpson's diversity index as well as non-parametric methods, such as abundance-coverage and incidence-coverage. Comparisons of individual sequences and their frequencies may be made within and between T cell populations and depicted graphically as pie charts or overlapping sets, with statistical calculations of repertoire relationships made using JMP Software, SAS Institute, Inc., Cary, NC. As each sequence is defined individually, any subtle differences in repertoire may be reported.

Importantly, CDR3 motifs may be revealed not only at the level of amino acid sequence but also in terms of the biochemical and biophysical properties of the amino acids therein. Taken together, these attributes will be used to examine the clonotypic composition of the naïve and memory CD4 and CD8 T cell pools to determine particular TCR signatures.

TCR sequencing will be performed prior to starting treatment, at count recovery after induction and before consolidation, at 8 weeks after the last round of consolidation, and at 1 year after therapy. Additional blood will not be drawn for this testing.

6.4.6 Cytokine Release Syndrome

We will measure cytokine release with DLI based on peripheral blood analysis. Blood will be taken on the day of cell infusion for both induction and consolidation 2, to be drawn before infusion and 2 days after infusion for both induction and consolidation 2 to evaluate for cytokine release syndrome with DLI, For subjects who do not receive their DLI during induction but rather following consolidation #1 and consolidation #2, blood will be drawn before infusion and 2 days after infusion to measure cytokine release. We will use about 4-8 teaspoons of blood drawn from a vein by a needle-stick or through a central line each time you have these blood tests.

6.4.7 Geriatric evaluation

Comprehensive geriatric assessment: Patients will be required to complete the CGA before induction and 8 weeks after the final round of cellular therapy. We have demonstrated previously that inpatient geriatric assessment and management has positively affected quality of life in the elderly cancer inpatient at no greater length of hospitalization or extra cost than usual care[39]. The CGA has been validated in CALGB studies in elderly patients with solid tumors (breast, colon, prostate cancers)[40]. The mean time to complete the assessment is 23 minutes. Most patients can complete the self-administered portion of the assessment without assistance, and there is no association between age or educational level and the ability to complete the assessment without assistance. Patients may require assistance from the research team for three items: 1) Blessed Orientation- Memory-Concentration (BOMC) test; 2) Timed Up and Go; and 3) Karnofsky performance status (KPS). The remainder of the CGA is either self-administered or administered by a research assistant if the patient requires assistance. The following domains of CGA will be measured: a) Functional Status: Activities of Daily Living, Instrumental Activities of Daily Living, Karnofsky Performance Rating Scale, Timed Up & Go, Number of Falls in Last 6 Months b) Comorbidity: Physical Health Section c) Cognition: Blessed Orientation-Memory-Concentration Test d) Psychological: Hospital

Anxiety and Depression Scale e) Social Functioning: MOS Social Activity Limitations Measure f) Social Support: MOS Social Support Survey: Emotional and Tangible Subscales, Seeman and Berkman Social Ties, and g) Nutrition: Body Mass Index, % Unintentional Weight Loss in the Last 6 Months.

Biologic Markers: Serum concentrations of the candidate cytokines (IL-6, IL-1, IL-2, TNF-α, and CRP) will be determined using Luminex MAP, which facilitates the simultaneous evaluation of multiple immune mediators with advantages of higher throughput, smaller sample volume, and lower cost, when compared to ELISA. All testing will be done in triplicates using the human 29-plex LINCO assay. For RNA extraction, total cellular RNA will be extracted from the leukemic cells using a commercial RNA purification kit; RNA quality will be determined with an A260/A280 ratio and capillary electrophoresis on an Agilent 2100 Bioanalyzer automated analysis system. For DNA microarray analysis, biotinylated cRNA synthesis will be generated with 10 micrograms of total cellular RNA. RNA will be analyzed on the Agilent 2100 Bioanalyzer and then hybridized onto a U133A oligonucleotide array, and thus will be stained, and washed according to an Affymetrix protocol. DNA chips are scanned with the Affymetrix GeneChip scanner, and the signals are processed to evaluate the standard RNA measures of expression. Of note, each gene on this chip is represented by 10 to 20 oligonucleotides, termed a “probe set.” The intensity of hybridization of labeled messenger RNA (mRNA) to these sets reflects the level of expression of a particular gene. Samples will be collected before induction, after induction and before consolidation, and at 8 weeks after completing final round of cellular therapy.

Samples will be collected before start of induction and at count recovery prior to consolidation 1.

7.0 EVALUATION CRITERIA AND DEFINITIONS

1. Standardized response criteria will be used following **National Comprehensive Cancer Network (**NCCN) recommendations found on the NCCN website: www.nccn.org.
2. GVHD will be graded with standard criteria (see Appendix II). Other toxicity will be graded as per the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (see Appendix III). Grade 3-4 will be reported.
3. Unacceptable toxicity will be defined as either:
    a. Grade III or IV aGVHD of the gut or liver or Grade IV aGVHD of the skin lasting > 7 days;

   b. Other grade IV CTCAE toxicity attributable to the DLI (e.g. infusion reaction, and distinct from grade IV toxicity attributable to chemotherapy) and lasting > 7 days
   c. Treatment-related mortality (TRM), keeping in mind that anticipated mortality from standard induction chemotherapy in the elderly ranges from 11% for subjects 55-65 years-old with ECOG 0 to 50% for subjects older than 75 years with ECOG 2 [1].
4. Disease-free survival (DFS) is the time from CR to relapse or death.
5. Overall survival (OS) is the time from start of therapy to either death or the last day of follow-up for that patient.
6. Event Free Survival (EFS) is the time from start of therapy to progression of AML or relapse or death.
7. Neutrophil recovery time is defined as the first of 3 consecutive days in which the ANC is >0.5 x 10^9/L
8. Platelet recovery is defined as the first of 3 consecutive days in which the platelet count is >20 x 10^9/L
9. PD1/PDL1/PDL2 expression (marker of T cell exhaustion)
10.

8.0 SUBJECT REGISTRATION

All subjects who meet eligibility criteria for this study and sign a consent form must be registered into Velos eResearch within 24 hours of consent by a member of the Division of Hematologic Malignancies and Cellular Therapy team. We also request an email to the study chair, Dr. Anthony Sung, atanthony.sung@duke.edu. The eligibility checklist must be completed and the IRB approved consent form signed before any protocol therapy may be started on the patient or donor.

9.0 TOXICITY MONITORING AND ADR REPORTING

The investigator is required by Federal Regulations to report adverse experiences (AEs) that occur through the duration of the study according to the appropriate procedures listed below. The investigator is required to notify, Duke University Health System (DUHS) Institutional Review Board (IRB) Office, and DUHS Comprehensive Cancer Center if an event is serious, unexpected, and related or possibly related to the study. All serious adverse events (SAEs) that occur up to 30 days after the last Donor Lymphocyte Infusion (DLI) will be reported. After 30 days, SAEs will be reported if thought related to treatment for the duration of the study.

Definitions: An **adverse** event is any new, undesirable medical experience or change of an existing condition that occurs during or after treatment, whether or not considered product-related.

A **serious** adverse event is any untoward medical occurrence that suggests significant hazard or side effects that result in the following:

- results in death
- is life-threatening (places the patient at immediate risk of death)
- requires or prolongs in patient hospitalization
- is disabling or incapacitating

- is a congenital anomaly/birth defect

Any SAE will be reported to the DUHS IRB within 24 hours.

An **unexpected** adverse event is any adverse event, the specificity or severity of which is not consistent with the current investigator brochure or consent form.

This study will utilize the NCI Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0 to determine the severity of the reaction for adverse event reporting. Only adverse events meeting criteria Grade 3 or greater will be collected. Laboratory abnormalities will only be captured if considered clinically significant. Any reactions that is reportable (serious, unexpected and related/possibly related to the study) must be reported using the Duke University Health System (DUHS) Institutional Review Board (IRB) Office SAE form. All other side effects and toxicities will be recorded in the patient's research chart or documented on the computer record for the patient.

Specific plan in the event of microbial contamination of infused product:
Product sterility testing will include a STAT gram stain which will have to be negative prior to release for infusion. A 14 day culture will be performed by our microbiology laboratory as per FDA requirements. In addition, per the SOP of sterility testing, cultures will be maintained for 5 days in our stem cell laboratory though typically the product is infused fresh the day of collection or the following day. In the event that a culture becomes positive, the lab notifies the treating physician immediately who in turn notifies the patient and the treating staff. Subjects will be evaluated within the same day of notification of a positive culture by the treating team for any infection related incidents and treated appropriately. The evaluation will include a minimum of physical examination, vital signs and blood culture.
In the event of this occurrence, this will be considered an SAE and reported in an expedited manner as above for other serious adverse events if there is a change in the health status of the patient or requiring initiation of antimicrobials.

Investigation plan in case of cellular microbial contamination:
In the event that a product has a positive culture, all reasonable efforts will be undertaken to detect the possible source. For potential donor source, donor notification and return to clinic or primary care physician for examination if there are any signs or symptoms of infection will be recommended. Examination of the chain of custody log will occur to ensure proper transit of the product and review of the exact procedure with the laboratory technician who performed the cell selection to investigate any potential difficulties that arose (tubing malfunction, bag leakage, etc).

10.0 DATA MANAGEMENT AND DATA SAFETY MONITORING

10.1 Data Management

10.1.1 Electronic Case Report Forms (eCRFs)
The eCRF will be the primary data collection document for the study. The eCRFs will be updated in a timely manner following acquisition of new source data. Only data managers are permitted to make entries, changes, or corrections in the eCRF.

An audit trail will be maintained automatically by our in-house Microsoft Access database. All users of this system will complete user training, as required or appropriate per regulations.

10.1.2 Data Management Procedures and Data Verification
Users of the electronic CRF will have access based on their specific roles in the protocol. Data managers have full read/write access; all other key personnel have read only access.

All data changes are tracked by an audit trail. Basic error checking is performed to ensure that legal values and dates are entered. Data managers do a full check of all data to maintain accuracy. The ability to make data modifications is based on the user's role on the study.

10.1.3 Study Closure
Following completion of the studies, the PI will be responsible for ensuring the following activities:

- Data clarification and/or resolution
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to the designated laboratories

10.2 Data Safety Monitoring
The DCI Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. As specified in the DCI Data and Safety Monitoring Plan, the DCI Monitoring Team will conduct routine monitoring after the third subject is enrolled, followed by annual monitoring of 1-3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

The Safety Oversight Committee (SOC) will perform annual reviews on findings from the DCI Monitoring Team visit and additional safety and toxicity data submitted by the Principal Investigator.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS and DCI leadership, the CPC, the Safety Oversight Committee (SOC), , the Principal Investigator, the Duke Clinical Trials Quality Assurance (CTQA) office or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, which may include but is not limited to the National Institute of Health, National Cancer Institute.. Every reasonable effort will be made to maintain confidentiality during study monitoring.

11.0 STUDY DESIGN AND ANALYSIS

11.1 Primary Objectives:

10.1.1 Safety: Evaluate the safety of HLA-haploidentical DLI combined with conventional induction and consolidation chemotherapy in terms of unacceptable toxicity as defined in section 7.0.

A maximum of 30 subjects will be consented for a maximum of 21 evaluable subjects accrued to this protocol over a period of 2 years. Safety will be evaluated by the number of subjects with unacceptable toxicity as defined by any of the following:

i. Grade III or IV aGVHD of the gut or liver or Grade IV aGVHD of the skin lasting > 7 days;

ii. Grade IV CTCAE toxicity attributable to DLI (e.g. infusion reaction, as opposed to Grade IV CTCAE toxicity from chemotherapy) and lasting >7 days

iii. Treatment-related mortality (TRM), keeping in mind that anticipated mortality from standard induction chemotherapy in the elderly ranges from 11% for subjects 55-65 years-old with ECOG 0 to 50% for subjects older than 75 years with ECOG 2 [1].

Simon's optimal two stage design will be applied. These rules permit the study to be closed early if there is evidence that the true unacceptable toxicity rate is $\geq$ 0.40. The stopping rules in the table below will also allow us to test the null hypothesis that the true toxicity rate is $\geq$0.40 against the alternative hypothesis that the true toxicity rate is $\leq$ 0.20. The Type I and II error rates for this design are 0.15 and 0.2, respectively. Given that the true toxicity rate is 0.40 (0.20), the probability of stopping the trial at Stage 1 to accept the null hypothesis is 0.7 (0.16). The significance level of this design is 0.15 and the power is 0.80.

Stage	Total Accrual	Close study/therapy if number of subjects with unacceptable toxicity is $\geq$
1	11	4 (45%)
2	21	7 (33%)

Rates of unacceptable toxicity will be calculated and monitored with the stopping rules in the table above. Toxicity in the other categories will be informally monitored and tabulated by Type and Grade, but they will not be used to determine tolerability of the procedure.

For purposes of toxicity monitoring, each patient will be followed for unacceptable toxicity until a toxic event occurs or until 8 weeks after last cell infusion, whichever comes first. Accrual will not be suspended while collecting data for the interim analyses. However, as soon as a boundary within a stage in the table above is crossed, the study will be closed to further accrual. This design and the above boundaries will constitute our stopping rules for safety.

11.1.2 Efficacy: Evaluate the efficacy of HLA-haploidentical DLI infusion combined with conventional induction and consolidation chemotherapy in terms of donor-recipient chimerism and microchimerism.

The assessment of efficacy will be conducted after collecting data for a maximum of 21 evaluable subjects. Our primary efficacy endpoint is the 1-year DFS rate following adoptive transfer. Using the chi-square test, we will test the null hypothesis is the CR rate will be ≤0.20 against the true hypothesis that 1-year DFS rate is >0.45. With 21 patients, we can test this with approximately 84% power with one-sided alpha level of 0.05. Additional efficacy endpoints include the CR rate, overall response rate (CR + partial response), OS, and count recovery. CR and overall response rates will be calculated with one-sided 90% confidence interval. DFS and OS will be estimated using the method of Kaplan and Meier. All statistical analyses will be conducted in conjunction with our co-investigator, Zhiguo Li, Ph.D., Assistant Professor of Biostatistics and Bioinformatics.

11.1.3 Geriatric assessment: Medians and frequencies will be used to describe the baseline characteristics of participants and performance on the CGA. Overall survival and associated 95% CI will be estimated as in Aim 1 and correlated with CGA measures. Cox proportional hazards models will be fit for each CGA measure as a predictor of OS, in unadjusted models and models controlling for age, gender, and ECOG score. We also will estimate the hazard ratios in adjusted Cox regression models for continuous variables. To assess the incremental impact of cognitive and physical function variables on predicting survival, we will use Integrated Discrimination Improvement as described for survival analysis by Chambless et al.[41] and implemented in the RiskPredictionParams SAS Macro (macro version 8, Chapel Hill, NC). The variables included in the model will be those associated with survival in our analysis and included in Kantarjian's predictive model for early mortality[42]. Internal cross-validation of the model will be assessed using 1000 bootstrap replicates implemented in the RiskPredictionParams SAS macro. Logistic regression analysis will be used to investigate relationships between baseline CGA and exploratory outcomes of 30-day mortality and CR. Competing risk modeling will be used to investigate a differential effect of impairment in CGA measures by cause of death[43].

For correlative studies, probe ID's on Affymetrix U133A gene chip have been identified for the candidate cytokines and include: IL-6 (211000,

2055207, 204864, 204863, 205945, 212196), IL-1 (39402, 205067, 10118, 208200, 201404, 216245), IL-2 (207849, 204116), TNF-α (211841, 211282, 210405, 211495, 211153, 207643, 207849), and CRP (207849, 204116). The mean value for each cytokine from the Luminex assay will allow us to identify patients with high or low levels of each cytokine, and relate them to genetic typologies. Correlations between the serum cytokine levels and mRNA gene expression level for the cytokines will be evaluated by Spearman correlation. Specifically, the range of genes will be related to the panel of cytokines. Statistical significance will be assessed controlling for Type-I error using Hochberg tests, which are less conservative relative to the more common Bonferroni corrections[44, 45]. In order to reduce the dimensionality of the problem further, in follow-up tests, we will assess if factorization of the cytokines is feasible (using Principal Components Analysis (PCA)), and, if so, the resulting components will be related to the genetic typologies. We note that skew will likely be present in the cytokine panel which can impact the factorization above. We will assess skew, and, if necessary, transform the variables individually to bring about approximate normality All analyses will be conducted at a two-sided a-level of 0.05 using SAS statistical software, version 9.2 (SAS Institute Inc., Cary, NC). We will use Spearman's correlation to assess the relationship between the biologic markers (serum cytokines and gene expression values) and clinical markers (each domain on the CGA and ECOG PS). While this work is exploratory and it could be argued that there is no need to adjust for multiple testing we will adjust p-values by the Hochberg method to control the Type-I error rate inherent in multiple testing[46]. All statistical analyses will be conducted in conjunction with our co-investigator, Zhiguo Li, Ph.D.

11.2 Secondary Objectives:

11.2.1 Evaluate incidence and severity of acute GVHD.

In addition to monitoring the rate of grade III-IV aGVHD of the gut or liver and grade IV aGVHD of the skin as in 10.1.1, we will also monitor overall rates of GVHD. Rates will be calculated with one sided 85% confidence interval.

11.2.2 Evaluate incidence and severity of other adverse events.

We will monitor other adverse events as defined in sections 7.0 and 9.0. Rates will be calculated with one sided 85% confidence interval.

11.2.3 Evaluate efficacy with respect to disease response and survival.

Additional efficacy endpoints include response rate, DFS, EFS, and OS. The response rate will be calculated with one sided 85%

confidence interval. DFS, EFS, and OS will be estimated using the method of Kaplan and Meier.

11.2.4 Evaluate immune recovery.

Immune reconstitution results will be tabulated and recorded. For the evaluation of recovery of immune function post-engraftment, we will use descriptive statistics of cytokine profiles, lymphocyte and NK enumeration and flow-based assays and make patient-specific plots of immune function parameters across time. These measurements will be performed prior to induction, prior to each round of consolidation, 8 weeks after the last cycle of consolidation, and every 3 months after treatment for up to 2 years. Specifically, we will:

1. monitor leukocyte subsets using flow cytometry:

- CD3+
- CD4+
- CD8+
- regulatory (CD4+, CD25+, CD62L+)
- cytotoxic/late memory (CD8+, CD57+, CD28-) and activated (CD8+, HLADR+) T cells
- naïve CD4+ T cells with L-selectin expression (CD4+, CD45RA+/CD45RO-, CD62L+)
- gamma-delta T cells
- B cells (CD19+, CD3-, CD16-, CD56-)
- natural killer cells (NK) (CD3-, CD16+/CD56+, CD107+)
- natural killer T cells (NKT) (CD3+, CD16+/CD56+, CD107+),
- plasmacytoid dendritic cells (DCs) (CD123+, CD11c-)
- myeloid DCs (CD123-, CD11c+)

2. quantifiy recent thymic immigrants by the presence of TCR rearrangement excision circles (TREC) performed by real-time quantitative-PCR of DNA collected from an isolated fraction of CD31 T cells

3. perform next generation sequencing of TCR gene rearrangements to analyze diversity in the TCR repertoires produced by the rearrangements of the variable region genes.

4. in vitro assays of NK function (measure CD16 expression, intracellular CD107a degranulation and cytokine secretions of IFNy and TNFa after 4 hour co-culture with an effector:target ratio of 2:1)

5. measure immunoglobulin levels as a function of B-cell activity and recovery

6. measure cytokine levels, particularly IL1, IL1-beta, IL2, IL6, IL8, IL15, IL21, IFN-gamma, TNF-alpha, TGF-beta, CRP

7. microchimerism monitoring to determine the level of donor cells (as well as the presence of any pre-existing maternal-fetal microchimerism)

8. KIR typing to determine the effect of KIR ligand mismatch

9. PD1/PDL1/PDL2 expression (marker of T cell exhaustion)

11.2.5 Evaluate hematopoietic recovery.

Time to neutrophil and platelet recovery will be reported using descriptive statistics. Time to neutrophil recovery is defined according to the Center for International Blood and Marrow Transplant Research (CIBMTR) reporting guidelines. This is defined as the date of the first of three consecutive laboratory values where the ANC ≧ 500/µl. If daily laboratory values were not obtained, ANC recovery is defined as three consecutive laboratory values drawn more than a day apart as long as the ANC remains ≧ 500/µl. The CIBMTR defines the time to platelet recovery as the date of the first of three consecutive laboratory values obtained on different days where the platelet count was > 20,000/µl without transfusion.

11.3 Subgroup analysis:
Subgroup analysis will be conducted based on age (55-70 vs >70), sex, WHO classification, relationship between donor and recipient, pre-existing maternal-fetal microchimerism in mother-child pairs, presence of post-infusion donor chimerism and microchimerism, HLA mismatch, and KIR-ligand mismatch.

12.0 ANTICIPATED TOXICITY OF DLI PROCEDURE

12.1 GVHD
Although GVHD is a common complication of DLI, given the absence of GVHD in a similar study by Guo et al.[6], we do not anticipate any GVHD in our study. This is because unlike a traditional transplant in which that patient engrafts with donor cells, which then go on to cause GVHD, we do not expect long-term engraftment, and therefore donor cells should not persist long enough to cause GVHD.

12.2 Infections
Although infections are a common complication of hematopoietic stem cell transplantation and donor lymphocyte infusion, we do not expect any of the procedures specifically associated with DLI to increase the risk of infection beyond that associated with induction and consolidation chemotherapy.

12.3 Infusion Reaction/Engraftment Syndrome

Patients may experience reactions to the infusion itself including fever, chills, skin rash, pulmonary infiltrates, diarrhea, hepatic dysfunction, renal dysfunction, or confusion. Any such reaction would be transient and we would respond by slowing the infusion as per 6.3.4. Steroids, tocilizumab, acetaminophen, and intravenous fluids may also be administered in the case of such a reaction.

12.4 Risk of sensitization

Infusion of haploidentical cells may result in alloimmunization of the donor; i.e., sensitization, which may make engraftment after allogeneic hematopoietic stem cell transplantation more difficult, if such a therapy were planned later. However, as an institution, we have considerable experience overcoming sensitization and alloimmunization in the allogeneic transplant situation, and we do not anticipate significant problems with engraftment should an allogeneic transplantation be needed in the future.

13.0 DRUG INFORMATION

13.1Cytarabine (ara-C)

13.1.1 Source and Pharmacology: Cytarabine for Injection USP, commonly known as ara-C, an antineoplastic for intravenous, intrathecal, or subcutaneous administration, contains sterile lyophilized cytarabine (1-β-D-Arabinofuranosylcytosine). Cytarabine is an odorless, white to off-white, crystalline powder which is freely soluble in water and slightly soluble in alcohol and in chloroform. Cytarabine is a synthetic nucleoside which differs from the normal nucleosides cytidine and deoxycytidine in that the sugar moiety is arabinose rather than ribose or deoxyribose.

13.1.2 Formulation and Stability: Cytarabine is rapidly metabolized and is not effective when taken orally; less than 20% of the orally administered dose is absorbed from the gastrointestinal tract. Following rapid intravenous injection of cytarabine labeled with tritium, the disappearance from plasma is biphasic. There is an initial distributive phase with a half-life of about 10 minutes, followed by a second elimination phase with a half-life of about 1 to 3 hours. After the distributive phase, more than 80% of plasma radioactivity can be accounted for by the inactive metabolite 1-β-D-Arabinofuranosyluracil (ara-U). Within 24 hours, about 80% of the administered radioactivity can be recovered in the urine, approximately 90% of which is excreted as ara-U. Relatively constant plasma levels can be achieved by continuous intravenous infusion. After subcutaneous or intramuscular administration of cytarabine labeled with tritium, peak plasma levels of radioactivity are achieved about 20 to 60 minutes after injection and are considerably lower than those after intravenous administration.

13.1.3 Supplier: Commercial Supply.

13.1.4 Toxicity: Because cytarabine is a bone marrow suppressant, anemia, leukopenia, thrombocytopenia, megaloblastosis, and reduced reticulocytes can be expected as a result of administration of cytarabine. The severity of these reactions are dose and schedule dependent. Cellular changes in the morphology of bone marrow and peripheral smears can be expected. Following 5-day constant infusions or acute injections of 50 to 600 mg/m^2, white cell depression follows a biphasic course. Regardless of initial count, dosage level, or schedule, there is an initial fall starting the first 24 hours with a nadir at days 7 to 9. Viral, bacterial, fungal, parasitic, or saprophytic infections, in any location in the body may be associated with the use of cytarabine alone or in combination with other immunosuppressive agents following immunosuppressant doses that affect cellular or humoral immunity. These infections may be mild, but can be severe and at times fatal.

13.1.5 Route of Administration: IV over 5 to 7 days.

13.2 Idarubicin

13.2.1 Source and Pharmacology: Idarubicin HCl Injection contains idarubicin hydrochloride and is a sterile, semi-synthetic, preservative-free solution antineoplasticanthracycline for intravenous use.

Idarubicin HCl is a DNA-intercalating analog of daunorubicin which has an inhibitory effect on nucleic acid synthesis and interacts with the enzyme topoisomerase II. The absence of a methoxy group at position 4 of the anthracycline structure gives the compound a high lipophilicity which results in an increased rate of cellular uptake compared with other anthracyclines.

13.2.2 Formulation and Stability: Idarubicin HCl Injection is a sterile, red-orange, isotonic parenteral preservative-free solution, available in 5 mL (5 mg), 10 mL (10mg) and 20 mL (20 mg) single-use-only vials. Each mL contains idarubicin HCl 1 mg and the following inactive ingredients: glycerin 25 mg and water for injection q.s. Hydrochloric acid is used to adjust the pH to a target of 3.5.

Pharmacokinetic studies have been performed in adult leukemia patients with normal renal and hepatic function following intravenous administration of 10 to 12 mg/m2 of idarubicin daily for 3 to 4 days as a single agent or combined with cytarabine. The plasma concentrations of idarubicin are best described by a two or three compartment open model. The elimination rate of idarubicin from plasma is slow with an estimated mean terminal half-life of 22 hours (range, 4 to 48 hours) when used as a single agent and 20 hours (range, 7 to 38 hours) when used in combination with cytarabine. The elimination of the primary active metabolite, idarubicinol, is considerably slower than that of the parent drug with an estimated mean terminal half-life that exceeds 45

hours; hence, its plasma levels are sustained for a period greater than 8 days.

13.2.3 Supplier: Commercial Supply.

13.2.4 Toxicity: Idarubicin is intended for administration under the supervision of a physician who is experienced in leukemia chemotherapy. Idarubicin is a potent bone marrow suppressant. Idarubicin should not be given to patients with pre-existing bone marrow suppression induced by previous drug therapy or radiotherapy unless the benefit warrants the risk.

Severe myelosuppression will occur in all patients given a therapeutic dose of this agent for induction, consolidation or maintenance. Careful hematologic monitoring is required. Deaths due to infection and/or bleeding have been reported during the period of severe myelosuppression. Facilities with laboratory and supportive resources adequate to monitor drug tolerability and protect and maintain a patient compromised by drug toxicity should be available. It must be possible to treat rapidly and completely a severe hemorrhagic condition and/or a severe infection.

Pre-existing heart disease and previous therapy with anthracyclines at high cumulative doses or other potentially cardiotoxic agents are co-factors for increased risk of idarubicin-induced cardiac toxicity and the benefit to risk ratio of idarubicin therapy in such patients should be weighed before starting treatment with idarubicin. Myocardial toxicity as manifested by potentially fatal congestive heart failure, acute life-threatening arrhythmias or other cardiomyopathies may occur following therapy with idarubicin. Appropriate therapeutic measures for the management of congestive heart failure and/or arrhythmias are indicated.

Cardiac function should be carefully monitored during treatment in order to minimize the risk of cardiac toxicity of the type described for other anthracycline compounds. The risk of such myocardial toxicity may be higher following concomitant or previous radiation to the mediastinal-pericardial area or in patients with anemia, bone marrow depression, infections, leukemic pericarditis and/or myocarditis. While there are no reliable means for predicting congestive heart failure, cardiomyopathy induced by anthracyclines is usually associated with a decrease of the left ventricular ejection fraction (LVEF) from pretreatment baseline values. Since hepatic and/or renal function impairment can affect the disposition of idarubicin, liver and kidney function should be evaluated with

conventional clinical laboratory tests (using serum bilirubin and serum creatinine as indicators) prior to and during treatment. In a number of Phase III clinical trials, treatment was not given if bilirubin and/or creatinine serum levels exceeded 2 mg%. However, in one Phase III trial, patients with bilirubin levels between 2.6 and 5 mg% received the anthracycline with a 50% reduction in dose. Dose reduction of idarubicin should be considered if the bilirubin and/or creatinine levels are above the normal range.

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14.0 APPENDIX

I. RECIPIENT SCHEDULE OF EVENTS TABLE*

TEST[1]	At entry	During Therapy and Recovery[2]	At Count Recovery after IND1 or IND2	After Finishing All Therapies[6]	Relapse
Weight (Epic)	X	Daily	As clinically indicated	At 8 weeks and q3months x 2 years	
Height (Epic)	X				
BSA Epic)	X	At the beginning of treatment	As clinically indicated		
PS (Epic)	X		As clinically indicated	At 8 weeks and q3months x 2 years	
Comprehensive Geriatric Assessment (Research)	X	-	-	At 8 weeks	
ABC / DIFF (Epic)	X	ABC daily and ABC with diff daily when WBC reaches 500/uL until ANC >1500/ul for 3 days	As clinically indicated	Every 2 weeks or as clinically indicated	
Serum BUN/Cr/CA/MG/PO/AST/ALT/Bili/TP/ ALB/K/NA/CL /CO3/Glu/CMV viral titre (Epic)	X	Weekly and as clinically indicated	As clinically indicated	At 8 weeks after last therapy and every 3 months for 2 years	
HLA antibody detection (serum preferred)	X		X	At 8 weeks after last therapy	
Serum quantitative Beta-HCG testing for females (Epic)	X	-	-	-	
Bone marrow aspirate and biopsy with appropriate special studies (i.e. restaging/disease evaluation) (Epic)	X	Day 14 after induction[5]	at hematologic recovery after induction (4-6 weeks)	-	
Cardiopulmonary Testing[3] (Epic)	X				
Immune Reconstitution Panel (Epic)	X	-	X	At 8 weeks after last therapy and every 3 months for 2 years	
Minimal Residual Disease (MDR)(Peripheral Blood) (Research)	X		X	At 8 weeks after last therapy and every 3 months for 2 years	X
Minimal Residual Disease (MRD) (Bone Marrow aspirate[4] (Research)	X		X (when BM bx is done to assess disease)		X

KIR typing (Research)	X				
NK lysis assay (Research[8])	X		X	At 8 weeks after last therapy	X
TEST[1]	**At entry**	**During Therapy and Recovery[2]**	**At Count Recovery after IND1 or IND2[6]**	**After Finishing All Therapies[7]**	**Relapse**
Microchimerism (Research)	X		X	At 8 weeks after last therapy and every 3 months for 2 years	X
TCR sequencing (Research)	X	-	X	At 8 weeks after last therapy	X
Biomarkers of frailty (Research)	X		X	At 8 weeks after last therapy	
PD1/PDL1/PDL2 expression (Research)	X		X	At 8 weeks after last therapy	X
Cytokine Release Syndrome (Research)		To be drawn before infusion and 2 days after infusion for IND1, IND2, and CON2 as applicable			
Toxicity monitoring (Grade 3-4) (Epic)		Daily	Qo wk	Qo wk till 8 weeks after last therapy and then q 4 weeks for 12 more weeks	
Acute GVHD monitoring (Epic)		Daily	Qo wk	Qo wk till 8 weeks after last therapy and then q 4 weeks for 12 more weeks	

[1] Tests at entry are recommended to be done within 1 week before initiating therapy; however if there are no sig. change in patient's status and tests are less than 4 weeks old then they are acceptable; exceptions include bone marrow aspirate and biopsy and cardiopulmonary testing (acceptable if within 8 weeks)
[2] Therapy and recovery refer to the period while the patient is receiving induction (IND1 or IND2) or consolidation chemotherapy until count recovery and discharge
[3] For cardiopulmonary testing, refer to section 5.1.
[4]If patient already had a bone marrow biopsy and this was not collected, ok to skip
[5] Per investigator discretion
[6] Count Recovery would be considered when the ANC count is >1000/ul
[7] If any subjects do not complete all the therapies (i.e. Induction 1/2, Consolidation 1 and 2), they will be followed for survival, but do not need to complete any listed assessments
[8]Any leftover cells at q3months after completing therapy will be used to run NK Lysis, TCR sequencing, and PD1/PDL1/PDL2 expression research samples. Additional blood will not be drawn for this testing.
*Other labs may be performed/followed per institutional policy

II. DONOR SCHEDULE OF EVENTS TABLE

Test	At Entry
History, physical (Epic)	X
ABC / DIFF, Serum BUN/Cr/CA/MG/PO/AST/ALT/Bili/TP/ALB/K/NA /CL/CO3/Glu/CMV viral titre (Epic)	X
Serum quantitative Beta-HCG for females (Epic)	X
CXR (AP/lateral), EKG (12 lead) (Epic)	X
FACT labs* (Epic)	X
KIR typing (Research)	X
Microchimerism (Research)	
TCR sequencing (Research)	X

*See appendix for standard FACT (Foundation for Accreditation of Cellular Therapy) labs. FACT labs must be drawn and final test results available within 7 days prior to infusion into the patient.

III. GVHD Grading Criteria

GvHD						
	Grade value for overall scoring:	*+0*	*+1*	*+2*	*+3*	*+4*
	Skin		Maculo-papular eruption over <25% of body area	Maculo-papular eruption over 25-50% of body area	Generalized erythroderma	Generalized erythroderma with bullous formation and often with desquamation
	Liver		Bilirubin ≥2-<3 mg/dl; SGOT 150-750 IU	Bilirubin ≥3-<6 mg/dl	Bilirubin ≥6-<15 mg/dl	Bilirubin ≥15 mg/dl
	Gut		Diarrhea >500-1000 ml/day	Diarrhea >1000-1500 ml/day	Diarrhea >1500-2000 ml/day	Diarrhea >2000 ml/day; or severe abdominal pain with or without ileus

Overall GvHD Grade						
Date Scored	*Grade value for overall scoring*	*Skin*	*Liver*		*Gut*	*ECOG Performance*
	I	+1 to +2	0		0	0
	II	+1 to +3	+1	And/or	+1	0 - 1
	III	+2 to +3	+2 to +3	And/or	+2 to +3	2 - 3
	IV	+2 to +4	+2 to +4	And/or	+2 to +4	3 - 4

IV Toxicity Summary Form

Toxicity	Grade 3	4	5
Allergy/Immunology			
Allergic reaction/hypersensitivity (including drug fever)	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis	Death
Cardiovascular (Arrhythmia)			
Ventricular arrhythmia	Symptomatic and requiring treatment	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)	Death
Cardiovascular/Arrythmia – Other Specify:	Symptomatic and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)	Death
Cardiovascular (General)			
hypotension	Requiring therapy and sustained medical attention, but resolves without sequelae	Shock	Death
Coagulation			
Thrombotic microangiopathy (e.g., TTP or HUS)	Evidence of RBC destruction with creatinine (>3 x ULN) not requiring dialysis	Evidence of RBC destruction with renal failure requiring dialysis +/- encephalopathy	Death
Gastrointestinal			
Diarrhea (Autologous subjects)	Increase of ≥ 7 stools/day, or incontinence, or need for parenteral support for dehydration	Physiologic consequences requiring intensive care; or hemodynamic collapse	Death
Stomatitis/ mucositis	Painful erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia	Death
Vomiting	≥6 episodes in 24 hours over pretreatment; or need for IV fluids	Requiring parenteral nutrition; or physiologic consequences requiring intensive care; hemodynamic collapse	Death
Hemorrhage			
Hematuria	Persistent gross bleeding or clots; may require catheterization or instrumentation, or transfusion	Open surgery or necrosis or deep bladder ulceration	Death

	Other (specify site)	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention	Death

Infection/Febrile Neutropenia [Enter type/species/site at bottom of this block]

	Infection (documented clinically or microbiologically) with grade 3 or 4 neutropenia (ANC < 1.0 x 10^9/L)	Present	Life-threatening sepsis (e.g., septic shock)	Death
	Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection)	Present	Life-threatening sepsis (e.g., septic shock)	Death
	Infection without febrile neutropenia	Severe, systemic infection, requiring IV antibiotic or antifungal treatment, or hospitalization	Life-threatening sepsis (e.g., septic shock)	Death

Infection Type: Species: Site:

Event Date	Toxicity	Grade 2	3	4	5

Neurology

	Neuropathy - Cranial		Present, interfering with ADL	Life-threatening, disabling	Death
	Neuropathy – Motor		Objective weakness interfering with ADL	Paralysis	Death
	Neuropathy – Sensory		Sensory loss or paresthesia interfering with ADL	Permanent sensory loss that interferes with function	Death
	Seizure(s)	Seizure(s) self-limited and consciousnes s is preserved	Seizure(s) in which consciousness is altered	Seizures of any type which are prolonged, repetitive, or difficult to control	Death

Pulmonary

	Carbon monoxide diffusion capacity (DLco)		≥25% - < 50% of pretreatment or normal value	<25% of pretreatment or normal value	Death
	Dyspnea (shortness of breath)		Dyspnea at normal level of activity	Dyspnea at rest or requiring ventilator support	Death

	Hypoxia		Decreased O_2 saturation at rest, requiring supplemental oxygen	Decreased O_2 saturation, requiring pressure support (CPAP) or assisted ventilation	Death
	Pneumonitis/pulmonary infiltrates	Radiographic changes and requiring steroids or diuretics	Radiographic changes and requiring oxygen	Radiographic changes and requiring assisted ventilation	Death

Renal/Genitourinary

	Creatinine		>3 – 6 x ULN	>6 x ULN	Death
	Renal failure		Requiring dialysis, but reversible	Requiring dialysis and irreversible	Death

Secondary Malignancy

	Secondary Malignancy - Other Specify type: (excludes metastatic tumors)			present	Death

VOD

	Weight gain		≥10% or as ascities	≥10% or fluid retention resulting in pulmonary failure	Death
	Bilirubin		>3 -10 x ULN	>10 x ULN	Death
	Hepatic pain		Severe pain; pain or analgesics severely interfering with ADL	disabling	Death

V. Comprehensive Geriatric Assessment

The Comprehensive Geriatric Assessment is provided as a separate attachment.

VI. FACT Donor Test Requirements

Testing for the following infectious disease is performed within 30 days of donation for PBSC, Bone Marrow, and Directd Granulocyte donation, and within 7 days for DLI, NK Cell donation, on blood samples drawn on stem cell or other cellular therapy. Donors donating over a time period in excess of 30 days will have these tests repeated every 30 days (or 7 days if DLI or NK Cell). Unrelated cord blood donors are tested by the cord blood bank at the time of procurement of the cord blood donation. This information is provided to the transplant center through the NMDP data systems.

The testing panel for autologous and allogeneic adult donors not on IVIG supplementation (within 6 months) of the donation is listed below.

1. Hepatitis B Surface Antigen (HBsAg)[1,2]
2. Hepatitis B Core Antibody (Anti-HBc)[1]
3. Hepatitis C Virus Antibody (Anti-HCV)[1]
4. HIV antibody (Anti-HIV 1/2/0)[1,2]
5. HIV1/2 Nucleic Acid Testing (NAT)[1,2]
6. Hepatitis C Virus NAT[1,2]
7. Hepatitis B Virus NAT[1,2]
8. HTLV I/II Antibodies Serum[1,3]
9. CMV Immune Screen (Anti-CMV total antibodies)[1,3]
10. CMV NAT-(if CMV positive Peds Only)[2]
11. Syphilis-Treponemapallidum antibody for initial screen
12. If Syphilis screen is reactive, a confirmatory test is performed using the RPR Card Test System
13. West Nile Virus NAT[1]
14. Trypanosoma Cruzi (Chagas Disease)
15. ABO/Rh (Two separate sample collections i.e. NTL Panel and day 1 collection type and screen)
16. Red blood cell antibody screen

[1]FDA Required testing

[2]FDA recommended. Obtain in all patients<6 months of age or on IVIG or unable to make endogenous antibody

[3]FDA required for products containing high WBC content (i.e. granulocytes, DLI, and mobilized peripheral blood)