CD8-depleted non-engrafting HLA antigen-mismatched unrelated donor lymphocyte infusion in patients with myelodysplastic syndrome and secondary acute myeloid leukemia

Protocol Number: 20042

Principal Investigator:	Hany Elmariah, MD, MS
IND/IDE Sponsor/IND Number:	17305
Biostatistician:	Dr. Jongphil Kim
Radiation Oncologist:	**N/A**
Pathologist:	**N/A**
Radiologist:	**N/A**
Medical Monitor (If Applicable)	**Pending**

Additional sites: N/A		
Role	**Site**	

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the Supporting Agency Terms. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed of their obligation to meet the above commitments.

Principal Investigator:_____ Print/Type Name

Signed:_____ Date:_____

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: CD8-depleted non-engrafting HLA-mismatched unrelated donor lymphocyte infusion in patients with myelodysplastic syndrome and secondary acute myeloid leukemia

Study Description: Phase 1b/2 study of CD8-depleted non-engrafting HLA-mismatched unrelated donor lymphocyte infusion in patients with myelodysplastic syndrome (MDS) after failure or intolerance of hypomethylating agents (HMAs) or untreated secondary acute myeloid leukemia (sAML)

Objectives:

1. Determine the maximum tolerated dose (MTD) of CD8-depleted non-engrafting HLA-mismatched unrelated donor lymphocyte infusion (NE-DLI) in patients with MDS or sAML

2. Estimate the response rate to therapy with CD8-depleted NE-DLI in patients with MDS who have failed therapy with HMAs or untreated patients with acute myeloid leukemia (AML) secondary to an antecedent hematologic disease (sAML)

Endpoints:

Primary Endpoint:

Determine the maximum tolerated dose (MTD) of $CD8^-$ depleted cells from HLA-mismatched unrelated donors infused after induction chemotherapy for patients with advanced MDS after HMA failure or untreated sAML

Secondary Endpoints:

- Overall response rate according to the International Working Group (IWG) (see Appendix B).
- Overall survival.
- Progression-free survival.
- Hematologic response/improvement by IWG 2006 criteria for MDS patients and the IWG 2003 criteria for AML (1, 2).
- Occurrence of adverse events.
- Unexpected transplant complications (eg, graft-versus-host disease [GvHD]).

Study Population: **Eligible patients** will have a MDS with progression or lack of response after at least 4 cycles of a HMA or sAML. HMA failure will be defined by the IWG criteria or intolerance to the drug (1)

Number of Patients: Expected 15 in the phase Ib cohort and 10 in the phase II cohort. 28 patients maximum. (The number of patients in the phase I cohort can be as high as 18 (6 patients for each dose level).)

Phase: Phase 1b/2

Description of Sites/Facilities Enrolling Participants:	H. Lee Moffitt Cancer Center – Single Site
Description of Study Intervention:	Eligible subjects will receive intensive cytotoxic induction chemotherapy with any standard of care cytarabine-based regimen. Twenty-four to 36 hours after chemotherapy cessation, subjects will receive NE-DLI infusion at a dose determined by their dose cohort in phase 1 or the recommended phase 2 dose in phase 2. Subjects will remain hospitalized to receive supportive care during the period of aplasia, and after being discharged from the hospital they will undergo weekly study visits until day 56 from DLI infusion for toxicity assessments
Study Duration:	3-4 years
Participant Duration:	12 months

1.2 SCHEMA

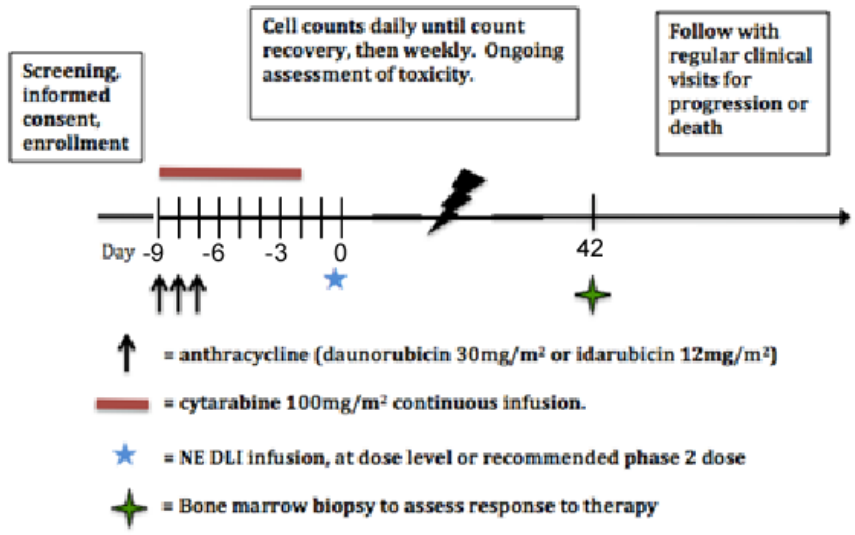


Figure: Example schema in conjunction with 7+3 Chemo. Note that the regimen will vary based on the specific chemotherapy regimen chosen by the provider.

1.3 SCHEDULE OF ACTIVITIES (SOA)

For explanation of procedures, please see Section 8 (Study Assessments and Procedures).

	Initial	Allowable time from start of induction	Starting Day 0	Day 28	Day 42	Day 56	6 months +/- 2 weeks	1 yr +/- 2 weeks
History and physical	X	Within 30 days			X	X	X	X
Performance status	X	Within 30 days						
Staging Bone Marrow Biopsy and aspirate for research	X	Within 30 days			X[a]			
Peripheral blood for research	X		**Daily days 0-7**		X[a]			
CBC & Diff	X	Within 7 days	ANC<500- 3x/week ANC≥500- weekly to day 56		X	X	X	X
CMP	X	Within 7 days	Weekly to day 56		X	X	X	X
CXR or Chest CT	X	Within 30 days						
Pregnancy test (women, childbearing age)	X	Within 30 days						
Human Immunodeficiency Virus	X	Within 30 days						
EKG	X							
MUGA or ECHO	X	Within 30 days						
HLA typing	X							
Anti-donor HLA antibody testing	X				X			
Adverse Event Monitoring			Weekly to day 56		X	X	X	X
GvHD evaluation			At onset, weekly until resolves		X	X		
Peripheral blood or bone marrow for donor chimerism					X[a]	X[b]		
CMV IgG	X	Within 30 days						
CMV PCR			Weekly through day 56					
C Reactive Protein	X	Within 7 days	Weekly through day 56					
Ferritin	X	Within 7 days	Weekly through day 56		X	X		

Abbreviations: ANC, absolute neutrophil count; CBC, complete blood count; CMP, comprehensive metabolic panel; CMV, cytomegalovirus; CXR, chest radiograph; CT, computerized tomography scan; diff, differential blood count; ECHO, echocardiogram; EKG, electrocardiogram; GvHD, graft-versus-host disease; MUGA, multigated acquisition; PCR,, polymerase chain reaction

Tracking of days begins from day of cell transfusion (eg, Day 56 is 56 days after initial cell infusion). Day of cell transfusion is marked as Day 0. Procedures can be performed ± 3 days from marked date, unless otherwise specified. [a]Specified day 42 samples may be performed prior to day 42 if the patient has recovered from aplasia, defined as ANC >500/μl. Otherwise, the specimen collections will be planned for day 42 +/-3 days.[b] Day 56 chimerism measurement may be omitted if donor T-cell chimerism is < 5% on first measurement or if whole blood/marrow chimerism is < 5% when T-cell chimerism is not measurable. If donor chimerism >5% on day 56, then the investigator will continue performing monthly GVHD evaluations and chimerism until donor T-cell chimerism decreases to <5% or until 1 year, whichever comes first.

Pre-treatment research samples may be added on to previously collected samples (e.g., Moffitt Total Cancer Care) in lieu of collecting new samples. Screening tests obtained as standard of care prior to patient consent to this trial may be used for screening purposes if the tests fall within the allowable time window to the start of induction chemotherapy as defined in the table above.

NOTE: In certain clinical circumstances (e.g. relapsed or terminally ill patients, risk of undue harm to patients, etc.) study tests may be omitted at the physician's discretion. Additionally, tests may be rescheduled as clinically indicated.

2 INTRODUCTION

2.1 STUDY RATIONALE

Patients with MDS after hypomethylating agent failure and patients with sAML are characterized by extremely poor prognoses.(3-5) These 2 patient groups are treated with cytotoxic induction chemotherapy, and have poor rates of overall survival. Survival is improved among patients who achieve a complete remission and are then treated with an allogeneic-bone marrow transplant (alloBMT), although this currently represents a minority of patients. This patient population is in critical need for new therapeutic options. The goal of this study is to use a novel non-engrafting CD8+-depleted DLI product to improve remission rates in patients with MDS with minimal toxicity, ultimately to increase the number of patients able to receive curative bone marrow transplant (BMT).

Several observations support the use of immunotherapy in treating MDS and sAML. AlloBMT, which elicits a graft-versus-tumor immune response, cures MDS and AML in a proportion of patients.(5, 6) In addition, immunosuppressive therapy can induce clinical responses in a minority of patients with MDS who have hypocellular bone marrow.(7-9) Analyses of responders to immunosuppressive therapies suggest that correction of T-cell dysregulation is correlated with clinical benefit.(10) There are anecdotal reports of MDS patients undergoing non-myeloablative alloBMTs who, despite losing their allografts, experience sustained responses.(11-13) MDS and AML are characterized by upregulation of markers of antitumor exhaustion such as programmed cell death protein 1 (PD1), PD1 ligand (PDL1), and cytotoxic T lymphocyte associated protein 4 (CTLA4) which is both predictive of hypomethylating agent (HMA) failure and exacerbated by HMA treatment.(14, 15) This suggests that strategies to reverse antitumor exhaustion should be effective in these diseases. Indeed, the therapeutic benefit of adoptive cellular therapies, such as DLI and microtransplantation, may result from this mechanism.(16-18)

We propose that a source of exogenous, immunocompetent, HLA-mismatched $CD8^+$-depleted $CD4^+$ DLI would be capable of reversing immune tolerance and eliciting a significant antitumor response in patients with MDS and sAML. $CD8^+$ depletion is intended to allow CD4+ help to be provided to exhausted host CD8+ T cells, many of which express T-cell receptors specific to tumor epitopes.(19, 20) If cytotoxic-competent donor CD8+ T cells are included, they may blunt the activity of host CD8+ T cells via an allogeneic mixed lymphocyte reaction.(21) Additionally, higher doses of CD8+ T cells would increase the likelihood of engraftment and, subsequently, lead to GvHD, morbidity, and mortality. Unlike other types of cellular therapy, this strategy would not require a specific antigenic target.

On the basis of prior literature, starting doses of 106 CD4+ T cells/kg (with <104 CD8+ T cells/kg) are likely to be well tolerated. Grafts containing <104 CD8+ T cells/kg do not cause severe GvHD, even among patients receiving lethal conditioning and no pharmacologic immunosuppression after transplantation.(22-24) The negligible risk of GvHD following CD8+ depletion permits the use of HLA-mismatched unrelated donors. By choosing HLA-mismatched, unrelated donors, we hope to induce more significant antitumor alloreactivity, and avoid recipient alloimmunization against potential HLA-matched BMT donors. This allows the treatment to be used in the pre-transplant setting. Additionally, eliminating HLA matching increases the donor pool, allows for a more readily available product, and lowers the cost of preparation.

NE-DLI has the potential to be an easily prepared and accessible product. Without the need for HLA matching, it has the potential to be used as a standard infusional product, similar to packed red cell or platelet transfusions. Ultimately, it is feasible that this could become a widespread therapy that is accessible to smaller medical centers, as well as tertiary and quaternary referral centers.

Initially, this therapy will be combined with induction chemotherapy to improve remission rates. If successful, the low expected toxicity could be a boon for affected patients, who are often frail and elderly. As NE-DLI will

ultimately be rejected by the recipient immune system, the therapeutic benefit may be temporary; therefore, the treatment is intended to serve as a bridge to consolidative BMT.

2.2 BACKGROUND

In 1966, Schwarzenberg, et al showed that transfusion of leukocytes could elicit remissions in patients with refractory acute leukemia.(25) Since that time, DLI has become a standard therapy, generally given after alloBMT to boost antitumor immunity and donor engraftment, but the therapeutic benefit is nearly offset by high rates of GvHD and mortality.(25-30) The effectiveness of DLI has provided clinical evidence that the immune system can be manipulated to successfully treat myeloid malignancies. Current investigations are, thus, aimed at harnessing antitumor immunity while decreasing toxicity.

We propose that a novel therapy consisting of exogenous, immunocompetent, HLA-mismatched CD8+ depleted CD4+ DLI for hematologic malignancies would be capable of eliciting a significant antitumor response without the concomitant toxicities of standard DLI.

2.2.1 CD4+ LYMPHOCYTES AND ANTITUMOR IMMUNITY

Antitumor immunity is a critical component in regulating the proliferation of cancer cells. $CD4^+$ T cells are the conductors of the immunologic response, coordinating effector components that include CD8+ cytotoxic T cells, macrophages, B cells and natural killer cells. CD4+ helper T cells are also the coordinators of immune memory,(31) and are essential for an effective and sustained immune response against cancer cells. Tumors evade the immune system and induce T-cell tolerance through multiple mechanisms, including decreased expression of immunogenic antigens,(32, 33) manipulation of cytokine balance in the tumor microenvironment,(34, 35) and upregulation of immune checkpoint molecules, such as programmed cell death protein 1, PD1 ligand, and cytotoxic T lymphocyte associated protein 4.(36)

In tumor-induced tolerance, the CD4+ helper T cells that recognize the tumor and initiate the host defense mechanisms become paralyzed.(37) Without CD4+ help, CD8+ T cells, though capable of recognizing antigens, fail

to remember the encounter and ultimately lose the capacity to produce cytokines or kill target cells. Thus, the CD8+ T cells themselves become exhausted.(38) A variety of tumor and viral infection experimental models have shown that the provision of allogeneic CD4+ T-cell help can prevent CD8+ T cells from becoming exhausted(39) and can reverse established exhaustion, enabling CD8+ T cells to respond appropriately.(40, 41) This restoration of help addresses the fundamental driver of tumor evasion and offers a potential foundation for therapy of all cancers and premalignant syndromes.

Among the strategies to restore antitumor immunity is allogeneic DLI. Although the mechanisms by which DLI stimulates an antitumor effect are not completely understood, there is evidence to support the effect is largely dependent on CD4+ T cells (Figure 1). Immunocompetent donor CD4+ helper T cells are capable of recognizing recipient HLA Class II antigen molecules as foreign, which initiates proliferation of alloreactive CD4+ T cells.(42-44) Once activated, donor T cells develop memory against the inciting antigen and initiate a signaling pathway mediated by antigen-presenting cells; this pathway is necessary to activate cytotoxic effector cells.(45) This process involves upregulation of CD40L ligand on the CD4+ T cells, which binds to CD40 on the antigen-presenting cell, (46, 47) and subsequent upregulation of CD70 on the antigen-presenting cell that interacts with CD27 on CD8+ cytotoxic T cells.(31, 48) The CD4+ T cells additionally release cytokines that further stimulate the immune response.(49) Meanwhile, CD8+ cytotoxic T cells recognize foreign antigens, such as tumor antigens, presented on HLA class I proteins and, with the stimulatory signals provided from the CD4+ T cells, become active against the tumor.(40, 41) Without effective CD4+ T cells, the potency of antitumor immunity is lost, but through these mechanisms, introduction of foreign CD4+ T cells are expected to reactivate the antitumor immune response.

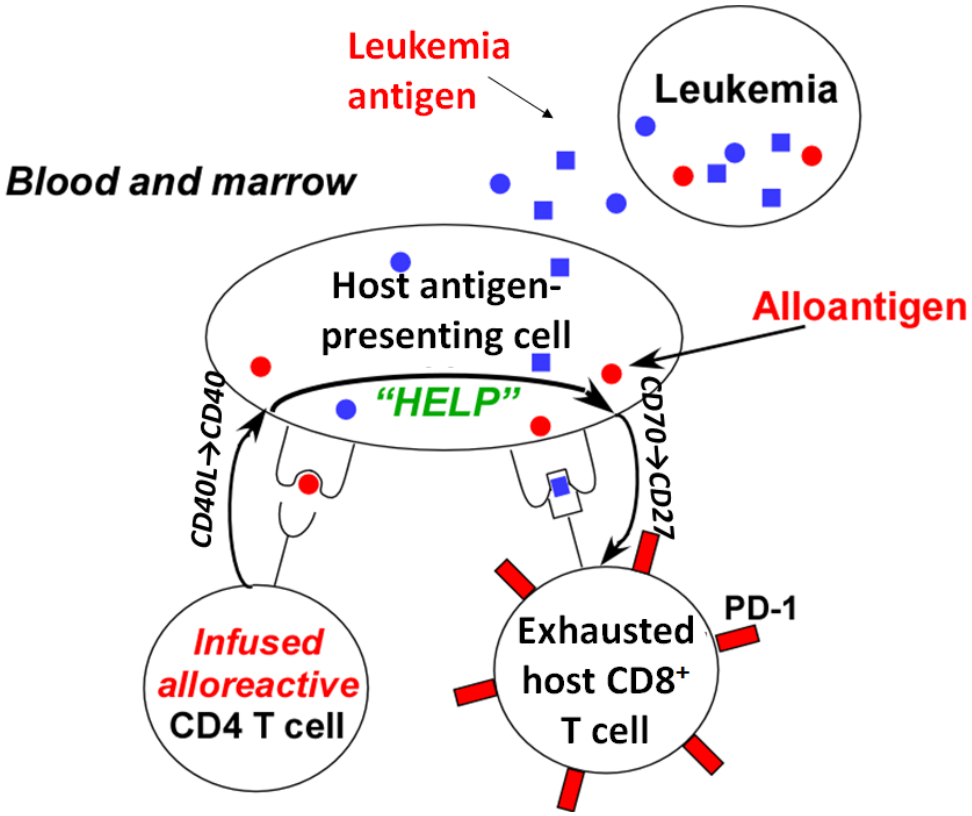


Figure 1: Schematic showing the role of CD4⁺ T helper cells in stimulating CD8+ T cells against leukemia antigens

The role of CD4⁺ T lymphocytes in antitumor immunity suggests that an infusion of donor CD4+ T lymphocytes may be able to promote a response in any CD8+ T cells able to recognize foreign antigens on the HLA class I antigen molecule. Theoretically, a donor CD4+ T cell could stimulate either a host- or donor-derived tumor-specific CD8+ T cell to create an antitumor effect. However, this mechanism also creates the problem of stimulating donor-derived host-specific CD8+ T cells against the host, thus precipitating GvHD. Indeed, in clinical practice, DLI leads to a potent boost in antitumor immunity known as graft versus leukemia (GvL), but the therapeutic benefit is nearly offset by high rates of GvHD and associated mortality.(25-30) Separating GvL and GvHD is a key problem in maximizing the benefit of DLI and other allogeneic immune therapies.

2.2.2 CD8⁺ DEPLETED DONOR LYMPHOCYTE INFUSION

The mechanism of DLI suggests that depletion of donor CD8⁺ T cells from the DLI product may preserve antitumor immunity while decreasing GvHD. Prior animal studies have shown that CD8⁺ depleted DLI creates an antitumor

effect and improved survival without concomitant GvHD.(24) That same study showed that $CD4^+$ $CD8^+$ DLI did cause GvHD, depletion of both $CD4^+$ and $CD8^+$ T cells led to a loss of antitumor immunity, and $CD4^-$ $CD8^+$ lymphocytes led to GvHD without an antitumor effect. Further, infusion of $CD4^+$ $CD8^-$ DLI in mice that lacked host $CD8^+$ T cells led to a decrease of the therapeutic effect of the DLI. The findings suggest that the antitumor effect of DLI requires donor $CD4^+$ and host $CD8^+$ lymphocytes, while donor $CD8^+$ cells are unnecessary and, indeed, detrimental, as they precipitate GvHD.

In humans, $CD8^+$-depleted DLI has been attempted in the post-transplant setting.(16, 23, 50) In a prospective study by Soiffer and colleagues, subjects with mixed chimerism were randomized to $CD8^+$ depletion, receiving a median of 0.7×10^5 $CD8^+$ cells/kg versus 32.0×10^5 $CD8^+$ cells/kg in the unmanipulated cohort.(23) There was less GvHD in the $CD8^+$ depleted cohort (0/9 versus 6/9), and incidences of relapse and mortality were similar in this small patient set. No other significant toxicities were reported. Hence, although sustained engraftment and resultant GvHD would be expected after DLI in the post-allogeneic transplantation setting, $CD8^+$ depletion negated the incidence of GvHD while retaining antitumor activity. In a review of $CD8^+$ depleted DLI cases in patients with relapsed chronic myeloid leukemia after BMT, response to therapy was most strongly correlated with the presence of host $CD8^+$ T cells infiltrating the marrow.(16) Further, clinical responses to $CD8^+$ depleted DLI were associated with down-regulation of genetic markers of exhaustion in the resident T-cell repertoire, consistent with the hypothesis that donor $CD4^+$ T cells provided help to reverse $CD8^+$ T-cell exhaustion.(16)

Although DLI is commonly used after transplant, there is limited experience using this therapy before transplantation.(51) Recently, investigators in China developed a technique called “microtransplantation” in which patients were infused with HLA -mismatched, granulocyte colony-stimulating factor stimulated allogeneic lymphocytes from related donors in conjunction with standard chemotherapy, resulting in only temporary levels of donor microchimerism (<1% donor).(17, 18, 52) In their initial trial, elderly patients with AML treated with induction chemotherapy and microtransplantation achieved significantly higher remission rates than those with standard induction (80% versus 43%), improved disease free survival, and no incidence of GvHD or increase in

infectious complications.(52) They subsequently showed that combining microtransplantation with standard consolidation improved overall survival and disease free survival in comparison with historical controls in low- and intermediate-risk AML patients.(17) Most recently, Hu et al applied the technique in patients with MDS or AML from antecedent MDS.(18) The MDS group achieved complete remission rates of 52% and 2-year overall survival of 85%, compared with complete remission of 36% and overall survival of 34% in the AML group. Again, no GvHD was observed. Some patients did experience infectious complications, though not significantly more than would be expected from chemotherapy alone. Although this microtransplantation technique did not involve $CD8^+$ depletion, the authors did report an increase in host $CD8^+$ T-cell response after treatment, suggesting the therapeutic mechanism may be reactivation of recipient-versus-tumor immunity through donor-cell help. Similar studies in the United States are ongoing and registered at clinicaltrials.gov.

At Johns Hopkins, 10 heavily pre-treated patients with hematologic malignancies and no validated therapeutic options were treated on protocol J0551. All patients received conditioning of cyclophosphamide 50 mg/kg/day IV for 2 days. The next day, they received an infusion of related HLA-haploidentical leukocytes, from which $CD8^+$ T cells had been removed. A dose of 1×10^5 $CD4^+$ cells/kg was infused into 5 patients and was well tolerated without a dose-limiting toxicity (DLT). There was 1 partial response in a patient with MDS. Three patients received 1×10^6 $CD4^+$ cells per kilogram without DLT. One of these patients achieved complete remission of refractory diffuse large B cell lymphoma. One patient received 1×10^7 $CD4^+$ cells per kilogram and had fatal cytokine release syndrome. Notably, that event occurred before widespread recognition of cytokine release syndrome and data to support new treatment options such as tocilizumab.(53) A final patient received 3×10^6 $CD4^+$ cells; the patient did not suffer any treatment-related toxicities but there was no disease response to the treatment. This unpublished work helped determine the MTD with this conditioning regimen and cell source, and demonstrated responses to the procedure.

In summary, $CD4^+$ T-cell help is necessary for a potent antitumor immune response. Exhaustion of $CD4^+$ T cells allows tumors to evade the immune system. Replacement of the exhausted $CD4^+$ T cells with immune competent

$CD4^+$ T cells from an allogeneic donor is expected to reignite the recipient's antitumor immune response without significant risk of developing GvHD.

2.2.3 STUDY DISEASES

2.2.3.1 MYELODYSPLASTIC SYNDROME

MDS is a clonal disorder of hematopoietic stem cells that results in defects in blood cell production and attendant morbidity and mortality.(54, 55) It is prevalent in older adults, (56) so low toxicity treatments are prioritized, although alloBMT is the only curative treatment modality. As MDS progresses, it has the potential to transform into AML, which is often managed aggressively with induction chemotherapy but portends a poor prognosis.(57)

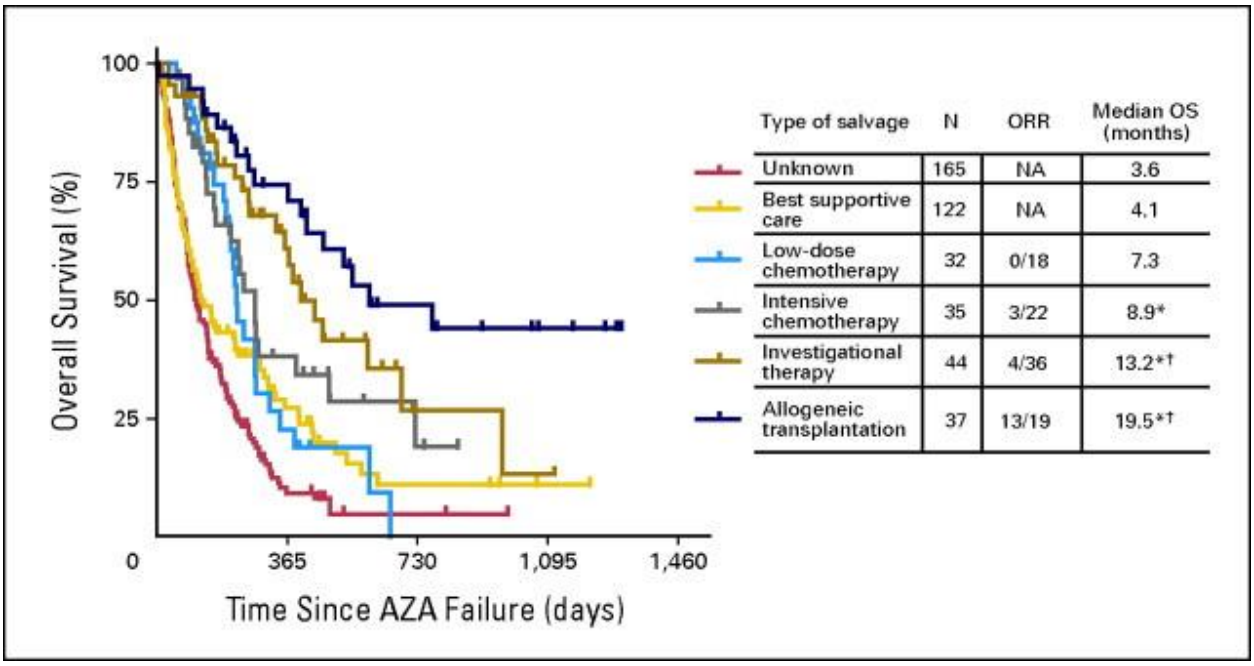
Management decisions in MDS are based on the Revised International Prognostic Scoring System (IPSS-R)[a],(58) which includes cell counts, bone marrow blast percentage, and cytogenetics of the malignant clone to determine risk of death or transformation to AML. Very low and low-risk patients are managed with observation, erythropoiesis stimulating agents, or transfusion support, and median survival is measured in years. Higher risk patients have a median survival of less than 2 years, and therapy directed against the malignant cells is typically indicated.(58)

The HMAS azacitidine and decitabine are the standard of care treatments in higher risk MDS. Both offer an improvement in rates of freedom from transfusion, and azacitidine has been shown to improve overall survival from 15 months to 24.5 months, compared to the investigators' preselection of best supportive care, low-dose cytarabine, or intensive chemotherapy.(59) Although DNA methyltransferase inhibitors mark an improvement over chemotherapy, only 50% of patients will experience a response,(1, 3-5, 59) and the median duration of response is 13.6 months. Clinical remissions in these studies last less than 4 months. The prognosis of patients who experience

[a] See appendix A

progressive disease on azacitidine or decitabine is especially poor, with median survival of less than 6 months without treatment.(5, 60)

Conventional AML-like induction chemotherapy is the standard of care for MDS patients who fail HMA therapy. This approach for patients with MDS after HMA failure can produce remissions, although infrequently, and survival is dismal. For example, Prebet et al showed intensive induction chemotherapy achieved complete remission in only 3 out of 22 patients failing azacitidine and in 2 out of 10 patients failing decitabine, and median survival was less than 9 months (Figure 2).(5) Other studies have similarly reported median overall survival (OS) typically less than 1 year once treatment is initiated.(57, 61, 62)



Type of salvage	N	ORR	Median OS (months)
Unknown	165	NA	3.6
Best supportive care	122	NA	4.1
Low-dose chemotherapy	32	0/18	7.3
Intensive chemotherapy	35	3/22	8.9*
Investigational therapy	44	4/36	13.2*†
Allogeneic transplantation	37	13/19	19.5*†

Figure 2: Overall survival outcomes for MDS patients after HMA failure by treatment modality as reported by Prebet, et al.(63)

As patients who do respond to induction chemotherapy achieve only a transient benefit, alloBMT is necessary to prevent relapse. This therapy is associated with the best outcomes in these patients, with reported median survival estimates as high as 19.5 months and long-term cure in approximately 40% of patients.(5) However, remission is typically required before BMT, which leaves the 80% of patients unable to achieve remission after HMA failure

ineligible for the procedure. Hence, novel therapies are needed to improve remission rates and make more patients eligible for alloBMT.

2.2.3.2 SECONDARY ACUTE MYELOID LEUKEMIA

AML is a clonal malignancy of myeloid cells characterized by an increase in abnormal, immature myeloid blasts and hematologic complications, including leukostasis, cytopenias, infections, bleeding, and death.(64) AML can arise de novo or through progression of an antecedent hematologic disorder (AHD), such as MDS or a myeloproliferative neoplasm. In patients diagnosed with MDS, the risk of progression to AML varies depending on severity of disease and cytogenetics, with high-risk patients progressing within months and low-risk patients progressing over many years.(58) As MDS is more prevalent in the elderly population, it follows that nearly 40% of AML cases in patients above 60 years of age are sAML arising from an antecedent disorder.(65)

Although MDS patients must be monitored closely for risk of progression to AML, many elderly patients with sAML first present after transformation, unaware of having had an AHD. The 2008 World Health Organization (WHO)classification system describes criteria to identify these cases.(66) By these criteria, antecedent MDS can be presumed on the basis of dysplasia of at least 50% of cells in at least 2 lineages, or presence of characteristic cytogenetic abnormalities, such as monosomy 5 and monosomy 7.(66) Although not included in the WHO criteria, subsequent studies have shown that somatic mutations in multiple genes, including SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR, or STAG2, are >95% specific to WHO-defined sAML.(67) The ability to identify sAML is important to guide treatment options and predict prognoses.

The treatment paradigm for sAML is similar to MDS after HMA failure, often using induction chemotherapy followed by consolidative BMT when possible. However, the prognosis is quite poor, particularly in elderly patients, due both to disease biology and an inability to tolerate aggressive treatment. In one retrospective study, 61 patients with sAML treated with induction chemotherapy had a 59% remission rate but only a 6.5 month median OS.(68) Another similar study evaluated outcomes of AML patients above 60 years of age who were

treated with induction chemotherapy.(65) The complete remission rate was 43%, although the median OS was only 107 days. This study included both patients with and without an AHD, but presence of an AHD was correlated with a worse prognosis. The discordance between remission rates and long-term survival in these studies highlights the fact that most of these patients will die from rapid relapse or treatment-related toxicities. For those patients with sAML who respond well to induction chemotherapy, alloBMT can yield 3-year survival rate as high as 40%.(69) Even in the elderly population who account for most sAML cases, OS rates at 3 years approach 40%.(70) These studies demonstrate both the dismal prognosis of patients with sAML and the potential for a fortunate few to achieve long-term survival with BMT. It is estimated that only 6% of elderly patients with AML are able to proceed to BMT because of early relapse, inability to achieve remission, or decline in functional status during induction.(71) Hence, novel treatments to increase the number of patients able to undergo BMT could dramatically improve the overall prognosis of this disease.

In summary, patients with MDS who have failed HMAs or progressed to sAML represent a population with an unmet clinical need. New treatment options are necessary to increase the number of patients who achieve complete remissions without significant treatment-related morbidity, thus making more patients eligible for curative BMT.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The most serious potential risks of the proposed treatment are death, cytokine release syndrome, and prolonged aplasia.

2.3.2 KNOWN POTENTIAL BENEFITS

Patients eligible for this study will be those with MDS after HMA failure and/or those with sAML. These groups lack effective treatment options. NE-DLI is expected to improve remission rates in these patients to increase their likelihood of reaching potentially curative allogeneic BMT.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Patients included in this study are those with aggressive myeloid malignancies who lack adequately effective treatment options. This study offers a novel therapeutic option that may improve their overall prognosis. The study is designed to minimize potential adverse effects of the therapeutic DLI. In addition to low starting doses of lymphocytes, safety is bolstered by the anticipated rejection of the infusion without engraftment. Although chemotherapy can allow persistence and activity of allogeneic cells, the intensity of AML induction chemotherapy is less than that which allows sustained donor chimerism.(72) Additionally, HLA mismatch increases the likelihood of graft rejection before engraftment(73), and $CD8^+$ depletion decreases the likelihood of engraftment.(74) Finally, cytokine release syndrome can be managed successfully with supportive care and tocilizumab.(53)

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Determine the MTD of $CD8^+$ depleted, non-engrafting, HLA-mismatched, unrelated donor lymphocyte infusion in patients with MDS or sAML.	The MTD of $CD8^+$ depleted cells from HLA-mismatched, unrelated donors infused after induction chemotherapy for patients with advanced MDS after HMA failure or untreated sAML.	This is a new therapy and the optimal dose is unknown. Thus, dosing is the primary endpoint as this improves safety for patients on the trial.
Secondary		
Estimate the response rate to therapy with $CD8^+$ depleted non-engrafting HLA-mismatched, unrelated donor lymphocyte infusion in patients with MDS who have failed therapy with HMAs or untreated patients with AML having transformed from an antecedent hematologic disease (sAML).	1. Overall response rate according to the IWG. 2 Overall survival 3. Progression-free survival 4. Hematologic response/improvement by IWG 2006 criteria for MDS patients and the IWG 2003 criteria for AML 5. Occurrence of adverse events. 6. Unexpected transplant complications (eg, GvHD).	These are clinically significant endpoints for evaluating the efficacy of treatments for MDS and AML.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a standard phase Ib/II trial design. All patients will be treated on a single arm. The objective of the phase I portion of the trial will be to determine the MTD of $CD8^+$-depleted peripheral blood cell leukocytes ($CD8^-$ PBCs)

when infused after cytotoxic chemotherapy. In the phase II portion of the trial, the efficacy of the treatment combined with cytotoxic chemotherapy will be determined. This is a phase 1b/2 study with dose escalation for determination of the MTD/recommended phase 2 dose (RP2D) of NE-DLI via a traditional 3+3 dose-escalation schema (see Appendix F) followed by an expansion cohort for assessing safety profile and preliminary estimation of the overall response rate (complete response and partial response based on IWG definition). Eligible patients will be treated with NE-DLI in the pre-transplantation setting.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Patients with MDS who have failed HMAs or progressed to sAML represent a population with an unmet clinical need. New treatment options are necessary to increase the number of patients who achieve complete remissions without significant treatment-related morbidity, thus making more patients eligible for curative BMT. We propose that a source of exogenous, immunocompetent, HLA-mismatched $CD8^+$-depleted $CD4^+$ DLI would be capable of reversing immune tolerance and eliciting a significant antitumor response in patients with MDS and sAML. $CD8^+$ depletion is intended to allow CD4+ help to be provided to exhausted host CD8+ T cells, many of which express T-cell receptors specific to tumor epitopes.(19, 20) If cytotoxic-competent donor CD8+ T cells are included, they may blunt the activity of host CD8+ T cells via an allogeneic mixed lymphocyte reaction.(21) Additionally, higher doses of CD8+ T cells would increase the likelihood of engraftment and, subsequently, lead to GvHD, morbidity, and mortality. Unlike other types of cellular therapy, this strategy would not require a specific antigenic target.

4.3 JUSTIFICATION FOR DOSE

For the dose-escalation portion, a minimum of 2 subjects and a maximum of 18 subjects will be enrolled. There will be 3 dose levels in the dose-escalation portion. MTD is defined as the highest dose level with the probability of DLT less than 33% (DLT events are defined in Section 6). The DLT observation window will be within 56 days following NE-DLI. If none of 3 patients experience a DLT, we will escalate to the next dose level. If one of the 3 patients experiences a DLT, we will enroll 3 additional patients and treat at the same dose level. In this case, if none of these additional 3 patients experiences a DLT, we will escalate to the dosage to next level. If 2 or more patients

among 6 enrolled patients experience a DLT, or 2 or more patients in the 3 patient cohort experience DLT, the dosage level exceeds the MTD and the previous dose level will be declared as the MTD. If the first dose level exceeds the MTD, the study will be suspended. If no subjects experience a DLT at any dose level, the MTD will be level 3 (5 x 10^7 CD4+ T cells/kg).

The RP2D is defined as the dose level selected to be evaluated in the expansion cohort or future phase II studies and will be equivalent to the MTD determined in the phase 1b portion of the study. Once we determine the RP2D level, we will enroll 10 additional eligible patients and treat with the RP2D level. The goal for this expansion cohort is to evaluate the safety and tolerability for patients who will be treated with NE-DLI in the RP2D dose level, and to evaluate the preliminary efficacy estimate for the ORR. Including the dose-escalation portion in which 3 or 6 enrolled patients will have been treated at the RP2D level, a total of at least 13 patients will be treated at the RP2D level. Based on previous data,(18) the complete response rate using microtransplantation was in the range of 36%-52% for MDS or AML from antecedent MDS patients. Hence, for the phase 2 portion, an ORR of 45% is considered clinically meaningful. With a sample size of 13 patients, if our ORR is 45%, the 95% CI (exact binomial) will be (18-74%); if the ORR is 50%, the 95% CI will be (22-78%).

4.4 END OF STUDY DEFINITION

The phase I portion of the study will continue until reaching the MTD to determine the RP2D. The phase II portion of the study will continue until an additional 10 patients are treated at the RP2D. The maximum total number of patients enrolled in this study will be 28 (6 per dose level plus 10 in the expansion cohort). We expect that the accrual rate will be one patient per every 5-6 weeks. With the DLI observation window (56 days after DLI), the dose-escalation portion may take up to a maximum of 2.5 - 3 years to complete and an additional 1.5 years for the expansion cohort.

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the SOA, Section 1.3.

The end of the study is defined as completion of the last visit or procedure shown in the SOA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Eligible subjects will have MDS and have failed treatment previously with HMAs or AML arising from an AHD (sAML), defined as meeting either item 1 or item 2 in the inclusion criteria below. All patients must fulfill item 3.

Eligible patients will be registered with the Clinical Research Office after signing the consent form. A registration may be cancelled in writing provided the subject has not begun protocol therapy.

1. MDS having failed HMA:
    a. Age 18-79 years, inclusive
    b. Pathologically confirmed MDS or myelodysplastic/myeloproliferative overlap (MDS/MPN)
    c. IPSS-R score intermediate, high, or very high[a]
    d. Must have failed therapy with an HMA (defined as lack of response by IWG response criteria(1) or intolerance of the drug).
2. sAML
    a. Pathologically confirmed AML according to WHO criteria.
    b. Evidence of an antecedent hematologic disorder (AHD) prior to acute leukemia including a known prior diagnosis of MDS, MPN, or MDS/MPN or data suggestive of an AHD such as cytopenias, fibrosis, macrocytic anemia, cellular or dysplasia at or prior to the time of diagnosis. If available, MDS-defining karyotypes (-7/del(7q), -5/del(5q), del(13q), del(11q), del(12p), t(12p), del(9q), idic(X)(q13), t(17p) (unbalanced translocations) or i(17q) (ie, loss of 17p), t(11;16)(q23;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36.3;q21), t(2;11)(p21;q23), inv(3)(q21q26.2), t(6;9)(p23;q34)) or somatic mutations in multiple genes including p53, TET2, JAK2, CALR, MPL, ASXL1, RUNX1, SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR, or STAG2 would also confirm eligibility.
    c. Age 18-79 years, inclusive
    d. May be previously untreated
3. For both cohorts:
    a. ECOG performance status of 0-2
    b. Deemed eligible to receive cytotoxic chemotherapy
    c. Creatinine clearance (CrCl)>50ml/min
    d. Total bilirubin <2 mg/dL (except for patients with Gilbert's disease), AST and ALT < 3x ULN
    e. Left Ventricular Ejection Fraction ≥ 50%

[a] See Appendix A

f. Willing and able to participate in study assessments

Enrollment will be open to all without prejudice.

5.2 EXCLUSION CRITERIA

Exclusion criteria:

1. Patients who have had systemic chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier. Hydroxyurea and/or targeted therapies (including but not limited to BCL2 inhibitors, JAK2 inhibitors, FLT3 inhibitors) during this period may be given as a bridging therapy to maintain disease stability while awaiting trial treatment. Intrathecal chemotherapy within this time frame is permitted. Intrathecal chemotherapy may be continued during protocol therapy in order to consolidate or maintain a central nervous system (CNS) remission, but not to treat active CNS disease
2. Acute promyelocytic leukemia, or the presence of t(15;17)
3. Patients receiving any other investigational agents
4. Uncontrolled concurrent illness including, but not limited to, ongoing and uncontrolled infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements
5. Pregnant women are excluded from this study because there is an unknown but potential risk for adverse events in the fetus. Breastfeeding should be discontinued if the mother is treated. These potential risks may also apply to other agents used in this study
6. Patients who have any debilitating medical or psychiatric illness that would preclude their giving informed consent or their receiving optimal treatment and follow-up
7. Patients with a poor functional status of ECOG 3-4, or otherwise deemed unfit to tolerate induction chemotherapy.
8. Patients with blastic transformation of chronic myelogenous leukemia are ineligible
9. Exposure to a humanized mouse chimeric antibody within 1 year of starting trial therapy, as this could sensitize patients to components of the CD8 depletion column that may be present in small amounts in the cell product
10. Prior allogeneic hematopoietic cell transplant.

5.3 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice, (pomelos, exotic citrus fruits, grapefruit hybrids, or fruit juices) from 1 day before the start of chemotherapy until after the final dose.
- Abstain from alcohol for 24 hours before the start of each dosing session until after collection of the final PK and/or pharmacodynamic sample.
- Abstain from use of tobacco products, as the use of nicotine-containing products (including nicotine patches) will not be permitted while they are in the clinical unit.
- Minimize interactions with household contacts who may be immunocompromised.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Screen failure individuals who do not meet the criteria for participation in this trial because of a reversible risk factor may be rescreened after resolution/treatment of the risk factor. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Eligible patients will be identified and offered the study during clinic visits and/or hospital admissions. Referrals to the trial are expected from outpatient Leukemia clinic and BMT clinic.

5.6 DONOR CRITERIA

5.6.1 DONOR ELIGIBILITY

I. Inclusion Criteria:

a. Age ≥ 18
b. Weight ≥ 110 pounds
c. HCT ≥ 30%
d. PLT > 100k
e. WBC ≥ 3.50k

II. Exclusion Criteria:

1. AB blood type
2. Positive testing for or known history of a communicable infectious disease
3. Unwilling or unable to provide informed consent
4. Has a medical contraindication to blood donation
5. Unwilling to donate during business hours, Monday-Friday
6. Not suitable for apheresis based on donor safety issues

5.6.2 DONOR RECRUITMENT

This trial will require unrelated subjects who are willing to donate blood to be used for NE-DLI, via either simple phlebotomy or mononuclear cell (MNC) collection by apheresis. Moffitt will contract with the New York Blood Center (NYBC) to use their existing donor pool and to collect the cells and ship to Moffitt for processing and infusion. In all cases, donor eligibility must be in compliance with 21 CFR 1271 and Foundation for the Accreditation of Cellular Therapy (FACT) standards. The donation procedure will be managed per institutional standard of care for apheresis/DLI donation and governed by the donor's procedural consent.

Recruitment: The New York Blood Center has an existing pool of volunteer donors who have agreed to be donors for blood product donations, either for standard of care or other clinical trials. The NYBC will invite donors from their existing pool to volunteer as donors for this trial. No donors with AB blood types will be recruited. Donors that respond to recruitment and agree to participate will undergo HLA testing and CMV testing, if not already performed as part of routine blood donation, and will be consented for possible cell collection. Because the desired donor requires only a class II HLA mismatch, HLA testing should include at least HLA DRB1, DQB1, and DPB1 as these are the markers required for donor selection.

Following consent, they will complete a health history questionnaire for eligibility as per FACT standards.

Potentially eligible donors chosen to donate will undergo routine FACT work up including physical exam within 6 months of cell donation and infectious disease testing and final donor clearance within 1 month of cell donation:

1. Infectious disease markers to rule out communicable infectious diseases.
    a. Positive results on infectious disease testing:
        i. Subjects will be informed of all positive results per standard procedures.
        ii. Mandatory reporting: Reportable diseases or conditioning will be reported to the State Department of Health based on the state of residence of the donor.
        iii. A proportion of potential donors likely will be determined to be ineligible based upon serologic infectious disease testing.
2. Donor clearance visit with a provider in the Moffitt Department of Blood and Marrow Transplantation/Cellular Immunotherapy or a provider at the contracted collecting institution including physical exam and review of systems and complete blood counts and comprehensive metabolic panel

Size of the potential donor pool: Based on the likelihood of finding HLA mismatched donors and the need for backup donors in case of unavailability of the preferred donor, the viability of this study will require a goal of at least 30 volunteer donors in the PDP. As experience with the conduct of this trial progresses, the target size may be adjusted upwards or downwards to reflect HLA patterns, donor suitability and sustainability, and on-call donor availability.

5.6.3 DONOR SELECTION

Donors will be chosen from a pool of previously HLA-typed healthy volunteers. Selection of donors will be

performed by the study PI.

1. The donor must satisfy criteria, including results of serologic testing for infectious diseases, established by the National Marrow Donor Program and the Foundation for the Accreditation of Cellular Therapies (FACT) for MNC, Apheresis, donation, and leukapheresis.

2. The donor may not be a first or second degree relative of the patient.
3. There must be at least one HLA Class II (HLA-DRB1, HLA-DQB1, or HLA-DPB1) antigen mismatch between the donor and recipient in the donor anti-recipient (graft-versus-host) direction.
4. The donor must not have a major ABO incompatible red blood cell type:
    a. If the recipient is blood type O, then the donor must be blood type O
    b. If the recipient is blood type A, then the donor must be blood type O or A
    c. If the recipient is blood type B, then the donor must be blood type O or B
    d. If the recipient is blood type AB, then the donor can be any blood type
5. If recipient is cytomegalovirus (CMV) seronegative, then a CMV seronegative donor will be selected.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Donors will have their blood collected via peripheral whole blood collection (450 ml into CPDA-1) or a leukapheresis procedure to collect mononuclear cells under steady state conditions (without mobilization). Each leukapheresis collection will be performed on a continuous flow cell separator (COBE Spectra or Spectra Optia, Terumo or equivalent device) using institutional standard operating procedures for MNC collection. The method of blood donation (phlebotomy versus leukapheresis) will be determined by estimating the volume of blood required to obtain the targeted CD4+ T-cell dose and based on standard institutional practices/preferences. Since the normal range of peripheral blood CD4+ T-cell counts is 0.5-1.5 x 10^6/ml, it is likely that simple phlebotomy will be sufficient for dose level 1 but leukapheresis will be required for dose levels 2 and 3. Based upon extensive prior experience, a 4-hour leukapheresis procedure should be sufficient to obtain 5 x 10^7 CD4+ T cells/kg of recipient ideal body weight (IBW). Target collections will be 30% more than the desired dose to accommodate for cell loss during the depletion process. Cells collected remotely will be shipped overnight to the administering site for processing and infusion.

The product will undergo CD8+ depletion in the Moffitt Cell Therapy Laboratory. All standard operating procedures will be followed. The product will be analyzed for nucleated cell count, viability, and surface expression of CD3, CD4, and CD8. CD8+ depletion will take place on the CliniMACS® Selection System (Miltenyi Biotec, Auburn, CA). The CD4 concentration will be used to calculate the patient dose. The calculated volume will be removed and prepared for infusion according to institutional standard operating procedures. The removed cells will either be

infused that evening or suspended in preservation medium (e.g., HypoThermosol, Biolife Solutions, Bothell, WA or similar) and infused the following day.

6.1.2 DOSING AND ADMINISTRATION

6.1.2.1 PRE-TREATMENT EVALUATION

All patients will require documentation of a detailed history and physical examination and standard evaluation of cardiac, liver and renal function. All patients will undergo a bone marrow aspirate and biopsy for morphological, cytogenetic and flow cytometric evaluation upon registration on protocol, along with other standard disease evaluations (e.g., CT of chest, abdomen, pelvis) where applicable. An echocardiogram or MUGA scan must be performed no more than one month prior to enrollment.

6.1.2.2 CHEMOTHERAPY

All subjects will receive any standard AML induction chemotherapy regimen with cytarabine backbone as chosen by the treating provider. Patients may receive the induction chemotherapy regimen in the hospital or in the outpatient setting as per standard practice for the specific regimen. All subjects must be admitted to the hospital prior to infusion of NE-DLI. Due the chemotherapy, there is an expected period of aplasia with associated severe neutropenia (ANC < 500/microliter) that typically last for at least 3 weeks from the start of chemotherapy. Subjects should remain in the hospital until aplasia resolves (defined as ANC > 500/microliter) or until at least day 28, whichever comes first. After neutrophil recovery and post-neutrophil recovery bone marrow biopsy, patients in remission may be treated with standard consolidation chemotherapy to maintain disease response.

Standard regimens include, but are not limited to:

Table: Examples of standard regimens for AML induction chemotherapy with cytarabine backbone.

Days, range	**Induction regimen,** Chemotherapy	**Dose and Administration**
	CLAG	
-5 to -1	Cladribine	5 mg/m^2/day IV
-5 to -1	Cytarabine	2 gm/m^2/day IV

-6 to -1	G-CSF	300mcg daily SQ
	7+3	
-7 to -1	Cytarabine	100 mg/m^2 IVCI x 7 days
-7 to -5	Idarubicin	12 mg/m^2 IVP x 3 days
	HiDAc, patient age < 60 y	
-5 to -1	Cytarabine	2 g/m^2 IV over 3 hours Q12H (10 doses)
	HiDAc, patient age > 60 y	
-5 to -1	Cytarabine	1 g/m^2 IV over 3 hours Q12H (10 doses)
Note: A more exhaustive list is available in the National Comprehensive Cancer Network Clinical Practice Guidelines for Acute Myeloid Leukemia (nccn.org). Standard regimens that incorporate FDA approved targeted therapies with cytarabine-based chemotherapy are permitted. However, concurrent use of investigational agents is not permitted.		

Because of cell procurement and processing requirements, the first day of chemotherapy must be timed so that the donor leukapheresis occurs on a Monday, Tuesday, Wednesday, or Thursday; the cell processing occurs on a Tuesday, Wednesday, Thursday, or Friday; and the cell infusion occurs on a Tuesday, Wednesday, Thursday, Friday, or Saturday. Because the donor cells are infused into the patient 24 to 36 hours after the completion of chemotherapy, the acceptable start days of chemotherapy vary by regimen.

In the case of a need to discontinue cytotoxic chemotherapy before infusion of cells, the patient will be taken off protocol because of toxicity. This will not be classified as a DLT in the phase 1 portion of the trial.

6.1.2.3 DONOR LYMPHOCYTE INFUSION

All patients will receive an infusion of mononuclear cells, apheresis products depleted of CD8+ T cells using the CliniMACS® system with CliniMACS® CD8 reagent. Products will be labeled as “T Cells, Apheresis: CD8 reduced.” The numbering of the dose levels is from the lowest to the highest cell dose. The first cohort of patients (dose level 1) will receive induction chemotherapy followed by an infusion of CD8+ T cell–depleted donor lymphocyte infusion from an intentionally HLA-mismatched unrelated donor at a dose of 1 x 10^6 CD4+ T cells/kg of recipient IBW. The

cells will be infused from 24-36 hours after the cessation of induction chemotherapy. If criteria for dose escalation are met (section 10.4), patients on dose level 2 and 3 will receive cells containing an intended dose of 1 x 10^7 or 5 x 10^7 cells/kg, respectively.

Dose level	CD4 T cells / kg
1	1 x 10^6
2	1 x 10^7
3	5 x 10^7

The CliniMACS® system with CliniMACS® CD8 reagent is a medical device that is used to enrich or reduce CD8+ T cells from human blood products. The CliniMACS® System intended for selection of CD8+ cells comprises 4 primary components: 1) CliniMACS® CD8 Reagent – colloidal superparamagnetic iron dextran beads linked to a murine antibody against human CD8; 2) CliniMACS®plus Instrument – a software controlled instrument that processes the blood sample (cell product); 3) CliniMACS® Tubing Set, (Standard or LS) – a single-use, sterile, disposable tubing set with 2 proprietary cell selection columns; and 4) CliniMACS® PBS/EDTA Buffer – a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the in vitro preparation of blood cells. The system utilizes magnetic cell sorting (MACS®), a powerful tool for the isolation of many cell types6, to selectively enrich or deplete the cell population of interest. In this case, CD8+ T cells are labeled with a monoclonal antibody linked to superparamagnetic particles and then are depleted from the blood product by passage through the CliniMACS® system, which incorporates a strong permanent magnet and a separation column with a ferromagnetic matrix to remove the labeled cells. **It is worth noting that the therapeutic agent in this trial, CD8+ T cell–depleted blood cells, comes out of the device and is not intended to contain any component of the device.**

Further information on the CliniMACS® system with CliniMACS® CD8 reagent can be found in the Investigator's Brochure: http://medbioscience.com/catalog/Miltenyi%20Biotec/CliniMACS/CliniMACS.pdf

Leukapheresis of an unmobilized donor is performed using the mononuclear cell setting within 48-72 hours prior to the scheduled time of infusion.

CD8 depletion is performed using the CliniMACS® magnetic cell separation system and the CliniMACS® CD8+ depletion reagent. Following CD8+ depletion, the cells are washed and pelleted. If not planned for immediate infusion (within 4 hours of CD8+ depletion), the cells will be resuspended to a concentration of 5 x 10^7 cells/ml in a preservation medium (e.g., HypoThermosol).

As cells will be collected remotely, cells will be shipped at 2-8 degrees Celsius overnight to the administering site. CD8+ depletion will be performed at the receiving site using the CliniMACS® magnetic cell separation system and the CliniMACS® CD8+ depletion reagent as described above.

Release testing of the CD8+ depleted product includes: 1) viability by tryptan blue dye exclusion and flow cytometry; 2) flow cytometry for CD3, CD4, and CD8 demonstrating that the number of CD3+CD8+ cells in the product is <3.2% the number of CD3+CD4+ cells; 3) sterility by gram stain.

6.1.2.4 INFECTION PROPHYLAXIS AND THERAPY

Antifungal prophylaxis will be administered according to institutional guidelines. Antifungal prophylaxis should begin no later than day 5 and continue until the absolute neutrophil count (ANC) is >500/microliter for 3 consecutive days (or for 2 consecutive measurements over a 3 day period) and the patient is free of evidence of infection. Antifungal prophylaxis can be substituted for patient intolerance or at the discretion of the treating physician.

Viral prophylaxis will be administered according to institutional guidelines. Standard options include, but are not limited to, valacyclovir (500mg po tid) or acyclovir (62.5 mg/m2 IV every 4 hours, if not tolerating oral medication) starting at day 1 of chemotherapy.

Antibiotic prophylaxis will follow institutional guidelines for induction therapy for acute myeloid leukemia/MDS. Antibiotics will be administered at least until the ANC is >500/microliter following induction.

Empiric or directed treatment of suspected or active infections will supersede prophylactic therapy.

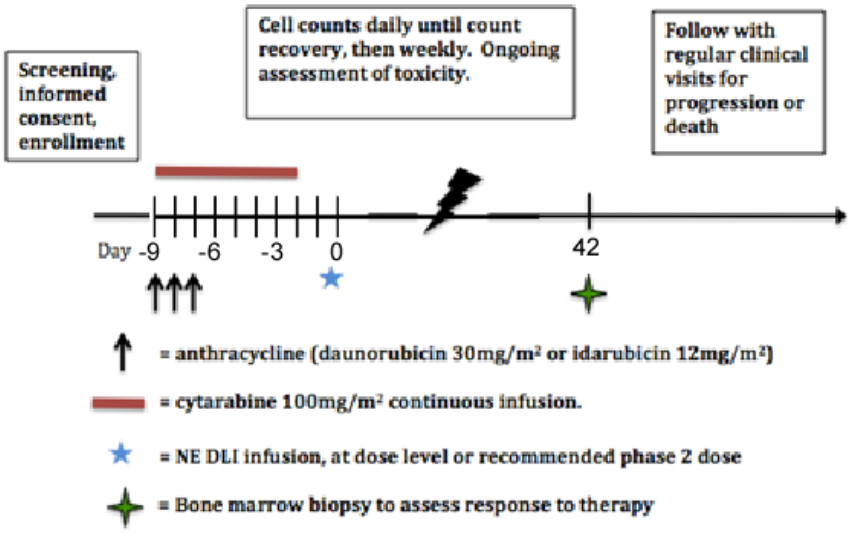
6.1.2.5 SUPPORTIVE CARE

Supportive care including fluids, anti-emetics, and tumor lysis management will be indicated according to institutional guidelines and the judgment of the treating physician.

6.1.2.6 TRANSFUSION SUPPORT

Platelet and packed red cell transfusions will be given per current institutional recommendations.

6.1.2.7 TREATMENT SCHEME



The figure shows the protocol schema as planned for patients undergoing standard 7+3 induction chemotherapy. The induction regimen may vary per judgment of the patient's provider.

6.1.3 DURATION OF THERAPY

Subjects are eligible for only one lymphocyte infusion. For the dose-escalation portion, a minimum of 2 subjects and a maximum of 18 subjects will be enrolled. There will be 3 dose levels in the dose-escalation portion. MTD is defined as the highest dose level with the probability of DLT less than 33% (DLT events are defined in Section 6). The DLT observation window is defined in section 9.4.2. If none of 3 patients experiences a DLT, we will escalate to the next dose level. If one of the 3 patients experiences a DLT, we will enroll 3 additional patients and treat at the same dose level. In this case, if none of these additional 3 patients experiences a DLT, we will escalate to the dosage to next level. If 2 or more patients among 6 enrolled patients experience a DLT, or 2 or more patients in the

3 patient cohort experience DLT, the dosage level exceeds the MTD and the previous dose level will be declared as the MTD. If the first dose level exceeds the MTD, the study will be suspended. If no subjects experience a DLT at any dose level, the MTD will be level 3 (5×10^7 CD4+ T cells/kg).

The RP2D is defined as the dose level selected to be evaluated in the expansion cohort or future phase II studies and will be equivalent to the MTD determined in the phase 1b portion of the study. Once we determine the RP2D level, we will enroll 10 additional eligible patients and treat with the RP2D level. The goal for this expansion cohort is to evaluate the safety and tolerability for patients who will be treated with NE-DLI in the RP2D dose level, and to evaluate the preliminary efficacy estimate for the ORR. Including the dose-escalation portion in which 3 or 6 enrolled patients will have been treated at the RP2D level, a total of at least 13 patients will be treated at the RP2D level. Based on previous data,(18) the complete response rate using microtransplantation was in the range of 36%-52% for MDS or AML from antecedent MDS patients. Hence, for the phase 2 portion, an ORR of 45% is considered clinically meaningful. With a sample size of 13 patients, if our ORR is 45%, the 95% CI (exact binomial) will be (18%-74%); if the ORR is 50%, the 95% CI will be (22%-78%).

The maximum total number of patients enrolled in this study will be 28 (6 per dose level plus 10 in the expansion cohort). We expect that the accrual rate will be one patient per every 5-6 weeks. With the DLI observation window, the dose-escalation portion may take up to a maximum of 2.5 - 3 years to complete and an additional 1.5 years for the expansion cohort.

6.1.3.1 DURATION OF FOLLOW-UP

Patients will be followed for a minimum of 56 days after DLI (day 0 is defined as the date of DLI infusion) and then until initiation of conditioning therapy for allogeneic stem cell transplantation, death, disease progression/relapse, or 1 year following the date of infusion, whichever occurs first. Patients removed from study for unacceptable adverse events or who develop treatment-related adverse events will be followed until resolution or stabilization of the adverse event. Patients who proceed to allogeneic stem cell transplantation will be removed from the study.

Post-infusion monitoring: Whole marrow and/or peripheral blood T-cell (CD3) donor cell chimerism will be measured after neutrophil recovery (ANC $\geq$ 500/μl) and no later than the day 42 sample collections to rule out engraftment. If donor chimerism is detected, this test will be repeated on day 56 and then monthly until graft rejection is confirmed. Non-hematologic toxicity will be assessed weekly while subjects are hospitalized, and weekly until day 42.

Disease assessment: Disease restaging requirements are designated in section 1.3.

In addition to disease assessments specified above, results of additional disease assessments performed as standard of care will be collected for study purposes until death or disease progression, whichever occurs first.

Off study criterion is the first event of the following:

- Death
- Disease progression
- One year after infusion (final study visit)

6.1.4 DEFINITION OF DOSE-LIMITING TOXICITY

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter 3 patients at the next dose level.
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose <highest dose administered>. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group experience DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤ 1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase II dose. At least 6 patients must be entered at the recommended phase II dose.

Dose-Expansion Cohorts

Once the RP2D is reached, a total of10 patients will be treated at this dose. For the expansion cohort, patients will continue to be monitored for occurrence of DLT. If 2 of the first 5 patients or if ≥ 2 of 6 patients experience DLT, the PI will discuss with all study investigators and with CTEP whether further addition of patients is needed to re-assess the RP2D.

Monitoring of safety and toxicity data will be captured in OnCore, Moffitt's clinical trials database.

Dose-limiting toxicities are defined as any of the following that is at least possibly attributable to the investigational treatment:

1. Grade 2-4 infusion toxicity
2. Grade 4 organ toxicity (as defined by NCI CTCAE v 5.0), not preexisting, not due to the primary malignancy, and not due to chemotherapy or infection
3. Grade 3 organ toxicity that does not resolve within 14 days and that is not preexisting, not due to the primary malignancy, and not due to chemotherapy or infection
4. Grade 2-4 acute GvHD
5. Delayed neutrophil recovery: ANC >500/μl does not occur within 56 days AND measurable donor chimerism is >5%
6. Grade 3-4 cytokine release syndrome (CRS) (see Appendix C) (75)
   Any grade 5 reaction other than progressive disease

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Donors will have their blood collected via peripheral whole blood collection (450 ml into CPDA-1) or a leukapheresis procedure will be performed to collect mononuclear cells under steady state conditions (without mobilization). Each leukapheresis collection will be performed using institutional standard operating procedures for MNC collection. The method of blood donation, phlebotomy versus leukapheresis, will be determined by prior to donation and based on the volume of blood required to obtain the targeted $CD4_+$ T cell dose.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

T Cells, Apheresis: CD8 Reduced and T Cells, Whole Blood: CD8 Reduced products are cell suspensions collected from allogeneic donors; the product is characterized by flow analysis (CD4+ cells and CD8+ cells). The product is

free of bacterial contamination as determined by gram stain analysis and is formulated in an infusion compatible solution. The T Cells, Apheresis: CD8 Reduced and T Cells, Whole Blood: CD8 Reduced products are provided as a fresh cell suspension. The product is a sterile suspension of mononuclear cells. If the cells will not be infused immediately, they may be suspended in preservation medium (e.g., HypoThermosol®). The volume in the bag will vary depending on the number of cells. Cell concentrations will be between $5x10^7$ cells/ml to $1x10^8$ cells/ml. The cells should be stored in a monitored 2-8°C refrigerator until ready for use.

6.2.3 PRODUCT STORAGE AND STABILITY

T Cells, Apheresis: CD8 Reduced and T Cells, Whole Blood: CD8 Reduced products are stored at 2-8°C until the time of use. The refrigerator units at clinical sites must be located in a controlled-access facility with an alarm monitoring system.

Products are shipped according to established SOPs, FACT Standards and DOT regulations. Fresh product shipments are monitored with single trip data logger to provide continuous temperature monitoring.

T Cells, Apheresis: CD8 Reduced and T Cells, Whole Blood: CD8 Reduced products should not be allowed to warm above 15°C during transport. A study was performed at Johns Hopkins University to evaluate the post-processing stability of T Cells, Apheresis: CD8 Reduced products (unpublished). Cell viability, as measured by trypan blue dye exclusion, was ≥88% for up to 72 hours after storage.

6.2.4 PREPARATION

T Cells, Apheresis: CD8 Reduced and T Cells, Whole Blood: CD8 Reduced products are cells that remain in an apheresis or whole blood product after depletion of CD8+ cells using the CliniMACS® system with the CliniMACS® CD8 reagent. The product is a sterile suspension of mononuclear cells suspended in HypoThermosol® or similar preservative. The volume in the bag will vary depending on the number of cells. Cell concentrations will be between $5x10^7$ cells/ml to $1x10^8$ cells/ml.

After manufacturing, the product is stored or shipped at 2-8°C prior to infusion. T Cells, Apheresis: CD8 Reduced and T Cells, Whole Blood: CD8 Reduced products should not be allowed to warm above 15°C during transport. Product infusion should follow established SOPs.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This is a single arm study. Patients will be treated in order of enrollment at the current dose level as delineated in the dose escalation schema. Neither the patients nor the trial physicians/providers will be blinded as all patients on trial will receive the same intervention. However, the final analysis will use de-identified raw data.

6.4 STUDY INTERVENTION COMPLIANCE

Data and safety monitoring will follow institutional protocol. Additionally, scheduled meetings will take place monthly and will include the protocol PI, clinical trial coordinator, and, when appropriate, the collaborators, subinvestigators, and biostatistician involved with the conduct of the protocol.

During these meetings the investigators will discuss matters related to: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives.

Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. Monitoring will be performed regularly by the MCC Clinical Monitoring Core for accuracy, completeness, and source verification of data entry, validation of appropriate informed consent process, reporting of SAEs, and adherence to the protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements. The Moffitt Protocol Monitoring Committee (PMC) meets monthly and reviews accrual, patterns and frequencies of all adverse events, protocol violations and when applicable, internal audit results.

6.5 CONCOMITANT THERAPY

Patients enrolled on this study may not be concurrently enrolled on another interventional therapeutic clinical trial.

6.5.1 RESCUE MEDICINE

The study site will supply the rescue medication tocilizumab for the management of cytokine release syndrome (CRS). This rescue medicine will be obtained locally. Although the use of rescue medications is allowable at any time during the study, the use of rescue medications should follow the algorithm for treatment of CRS as described in Appendix C. The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication must be recorded.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from NE-DLI does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

The data to be collected at the time of study intervention discontinuation will include the following:

- Cause of discontinuation
- Most recent laboratory values
- Evidence of adverse events
- Disease status at the time of discontinuation

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Patients may elect to withdraw from the study at any point without any further justification.

Additionally, patients may be removed from the study at the discretion of their medical providers on the basis of the following criteria:

- Adverse events or any concern that continuing in the study would be harmful
- Need for treatment not allowed in the study, including disease relapse while on study
- Failure by a patient to comply with study protocol
- Onset of pregnancy after enrolling in the study
- Cancellation of the study

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and if study site staff is unable to contact the participant.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit for 1 month and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, by making 3 telephone calls and, if necessary, by sending a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- A minimum of 2 years should be spent attempting to locate the patient. If after that period of time the coordinator has sufficiently documented all failed attempts to locate the patient, including sending a certified letter with no response, then he or she will be considered to have withdrawn from the study for the primary reason of being lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SAFETY ASSESSMENTS

Safety assessments will be as follows:

- History and physical: Initial and repeated at days 42 and 56, 6 months, and 1 year to evaluate for subjective or exam findings consistent with AEs.
- Bone marrow biopsy: Day 42 to evaluate the efficacy of the intervention. Day 42 bone marrow biopsy may be performed earlier than the day 42 if the subject has recovered from aplasia, defined as recovery of the ANC >500/μl.
- Peripheral blood and bone marrow chimerism: To rule out donor engraftment and confirm rejection of the DLI product, donor cell chimerism will be measured after neutrophil recovery (ANC $\geq$ 500/μl) or on day 42 +/- 3 days after infusion. Engraftment is necessary for GVHD, so product rejection increases the long term safety of the product.
- Complete blood count (CBC): 3 times per week until ANC recovers to 500. Weekly through day 56. This assessment is necessary to monitor for hematologic toxicity.
- Comprehensive metabolic panel (CMP): Weekly through day 56 to evaluate for toxicity to the liver, kidneys, or electrolyte derangements.

- C Reactive Protein (CRP) and Ferritin: Weekly through day 56 to screen for evidence of cytokine release syndrome (CRS)
- Cytomegalovirus polymerase chain reaction (PCR): Weekly through day 56 to monitor for CMV reactivation while awaiting immune reconstitution.

	Initial	Allowable time from start of induction	Starting Day 0	Day 28	Day 42	Day 56	6 months +/- 2 weeks	1 yr +/- 2 weeks
History and physical	X	Within 30 days			X	X	X	X
Performance status	X	Within 30 days						
Staging Bone Marrow Biopsy and aspirate for research	X	Within 30 days			X[a]			
Peripheral blood for research	X		**Daily days 0-7**		X[a]			
CBC & Diff	X	Within 7 days	ANC<500-3x/week ANC≥500-weekly to day 56		X	X	X	X
CMP	X	Within 7 days	Weekly to day 56		X	X	X	X
CXR or Chest CT	X	Within 30 days						
Pregnancy test (women, childbearing age)	X	Within 30 days						
Human Immunodeficiency Virus	X	Within 30 days						
EKG	X							
MUGA or ECHO	X	Within 30 days						
HLA typing	X							
Anti-donor HLA antibody testing	X				X			
Adverse Event Monitoring			Weekly to day 56		X	X	X	X
GvHD evaluation			At onset, weekly until resolves		X	X		
Peripheral blood or bone marrow for donor chimerism			If suspected GvHD		X[a]	X[b]		
CMV IgG	X	Within 30 days						
CMV PCR			Weekly through day 56					
C Reactive Protein	X	Within 7 days	Weekly through day 56					
Ferritin	X	Within 7 days	Weekly through day 56		X	X		

Abbreviations: ANC, absolute neutrophil count; CBC, complete blood count; CMP, comprehensive metabolic panel; CMV, cytomegalovirus; CXR, chest radiograph; CT, computerized tomography scan; diff, differential blood count; ECHO, echocardiogram; EKG, electrocardiogram; GvHD, graft-versus-host disease; MUGA, multigated acquisition; PCR,, polymerase chain reaction

Tracking of days begins from day of cell transfusion (eg, Day 56 is 56 days after initial cell infusion). Day of cell transfusion is marked as Day 0. Procedures can be performed ± 3 days from marked date, unless otherwise specified. [a] Specified day 42 samples may be performed prior to day 42 if the patient has

recovered from aplasia, defined as ANC >500/μl. Otherwise, the specimen collections will be planned for day 42 +/-3 days. [b]Day 56 chimerism measurement may be omitted if donor T-cell chimerism is < 5% on first measurement or if whole blood/marrow chimerism is < 5%. If donor chimerism >5% on day 56, then the investigator will continue measuring monthly until it decreases to <5% or until 1 year, whichever comes first.

Pre-treatment research samples may be added on to previously collected samples (e.g., Moffitt Total Cancer Care) in lieu of collecting new samples. Screening tests obtained as standard of care prior to patient consent to this trial may be used for screening purposes if the tests fall within the allowable time window to the start of induction chemotherapy as defined in the table above.

NOTE: In certain clinical circumstances (e.g. relapsed or terminally ill patients, risk of undue harm to patients, etc.) study tests may be omitted at the physician's discretion. Additionally, tests may be rescheduled as clinically indicated.

8.2 OTHER ASSESSMENTS

Baseline evaluations to be performed within 30 days of initiating treatment:

- Performance status: to confirm ECOG $\leq$2
- Chest X-ray or Chest CT: To rule out active pulmonary infection
- EKG and either MUGA or echocardiogram: To confirm adequate cardiac function
- HIV testing: To rule out active HIV infection which would require treatment prior to initiating chemotherapy
- Pregnancy testing: To rule out pregnancy as participation in this study may be dangerous to the fetus
- Bone marrow biopsy: To confirm diagnosis and evaluate baseline disease status
- CMV IgG: To allow for optimal, CMV matched donor selection

Baseline evaluations to be performed anytime prior to enrollment:

- HLA typing with anti-donor HLA antibody testing: To determine if this treatment increases production of anti-HLA antibodies.

Baseline evaluations to be performed within 7 days of initiating therapy:

- CBC: To confirm adequate hematologic parameters to proceed on study
- CMP: To confirm adequate kidney/liver function to proceed on study
- CRP and Ferritin: To risk stratify risk of cytokine release syndrome prior to treatment

- CMV PCR: To rule out active CMV infection and assist with donor selection

NOTE: In certain clinical circumstances (e.g. relapsed or terminally ill patients, risk of undue harm to patients, etc.) study tests may be omitted at the physician's discretion. Standard of care tests may be rescheduled as clinically indicated.

8.3 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES OR OTHER ENDPOINTS

Specimen Type	Baseline (Pre-treatment)	Days 0-7*	Day 42**
Peripheral blood sample	X	Daily	X
Bone marrow biopsy/aspirate	X		X

* Research sample collection may be omitted if the day falls on a weekend or holiday.

**May be obtained earlier than day 42 if ANC recovery to >500/uL. (See study calendar)

8.3.1 PLANNED CORRELATIVE STUDIES

Below are outlined correlative studies to be completed from the tissue samples. These will be performed as funding permits. Specific reagents and devices may be substituted for equivalent reagents/devices as available to the performing laboratory.

Hypothesis: In patients with MDS/AML, allogeneic HLA-mismatched CD4+ DLI will result in reversal of immune exhaustion of recipient tumor specific CD8+ T cells.

Aim 1: Determine if treatment of MDS/AML with HLA-mismatched CD4+ DLI results in reversal of expression of protein markers of immune exhaustion in recipient cytotoxic T cells.

Markers of Immune Exhaustion

Immune exhaustion is characterized by CD8+ T cell surface expression of inhibitory proteins such as PD-1, which contribute to treatment failure in MDS.(14, 36, 76-78) Thus, we wish to assess whether HLA-mismatched CD4+ DLI can reverse expression of negative immune checkpoints on exhausted CD8+ T cells in patients with MDS who failed standard therapy. All enrolled patients will have peripheral blood and bone marrow samples obtained at baseline prior to protocol chemotherapy, and at the time of hematologic recovery, 30-50 days after HLA-mismatched CD4+ DLI. Peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (BMMCs) will be enriched using standard Ficoll-Hypaque methods. The cells will be stained with propidium Iodide/acridine orange and live cells will be counted using a Cellometer 2000 automated cell counter (Nexcelom Biosciences, Lawrence, MA). The cells will be suspended in Recovery Freeze Medium (Invitrogen, Carlsbad, CA) and controlled rate frozen to -80°C before transfer to liquid nitrogen storage. In batch, baseline and post-treatment samples will be thawed, followed by isolation of CD4+ and CD8+ populations using MultiSort magnetic MicroBeads (Miltenyi Biotec, Gaithersburg, MD). For this CD4+ cells will be bead captured on one column and the flow through will be mixed with CD8 beads and then captured on a second column. Total RNA will be extracted from one million each of the isolated

CD4+ and CD8+ cells and stored for subsequent RNAseq analysis. The collected samples will be analyzed via multicolor flow cytometric assay to characterize expression of markers of immune exhaustion (TIM3, LAG-3, PD1, PDL1, CTLA4).(79-81) Pre-treatment and post-treatment values will be compared to determine if expression of exhaustion antigens is down regulated after HLA-mismatched CD4+ DLI therapy, thus predicting that immune exhaustion is reversed. The one-sample t-test will be used to assess if the markers are significantly changed after treatment.

Host versus Donor Origin of CD8+ T Cells

Because we hypothesize that reversal of immune exhaustion will specifically occur in the recipient's cytotoxic T cells, we wish to identify if reinvigorated CD8+ T cells are derived from the donor or the host as donor derived CD8+ T cells would be expected to lead to engraftment and potentially GVHD. Since the CD4+ DLI is intentionally HLA mismatched, RNA sequencing can be used to distinguish and quantify donor versus recipient HLA types in the samples.(82) We will isolate the tumor infiltrating cells in the marrow after HLA-mismatched CD4+ DLI and use RNA sequencing of HLA to determine if the cells are donor- or host-derived. We will supplement this testing with standard clinical bone marrow chimerism studies. In combination, these tests will assess whether donor CD4+ T cells are rejected and will confirm that the immune competent CD8+ T cells are of host origin.

Aim 2: In patients with MDS/AML who received HLA-mismatched CD4+ DLI therapy, characterize anti-leukemic activity and specificity of recipient marrow CD8+ T cells in response to HLA-mismatched CD4+ DLI.

Leukemia-Specific Cytolytic Activity of CD8+ T cells

After treatment with HLA-mismatched CD4+ DLI, we anticipate a reversal of immune exhaustion and a subsequent host-versus-leukemia response. We will evaluate changes to cytolytic activity of the recipient cytotoxic CD8+ T cells before and after therapy, first, in cytotoxicity experiments at different effector:target (CTL:tumor cell) ratios using leukemia blasts collected from the pre-treatment samples. If the frequencies of tumor-reactive T cells required more sensitive methods, we would use ELISPOT testing. Specifically, this sensitive assay can quantify a successful response to HLA-mismatched CD4+ DLI characterized by an increase in the frequencies of CD8+ T cells secreting interferon-γ (IFN-γ) in response to cognate tumor antigen, using peripheral or BM malignant cells as targets.(83) Cytotoxicity and/or ELISPOT results will be compared between patients who achieve a remission and those who do not to show that clinical response is dependent on marrow infiltration by activated cytotoxic T cells. The Satterthwaite t-test will be used to detect a significant difference in changes to cytolytic activity and increase in frequency of CD8+ T-cells before and after treatment, and between patients who achieve a remission and those who do not. The odds ratio will be estimated along with 95% confidence intervals by using the simple logistic regression or the generalized estimating equation (GEE) model. Building multivariable model will not be considered due to the small sample size issue. The data transformation (e.g., log2-transformation) may be considered to meet the normality assumption for the application of parametric analytic tools. The Wilcoxon singed-rank test or Wilcoxon rank-sum test may be considered as the secondary approaches if necessary. As this is an exploratory study, the multiplicity adjustment is not considered.

Clonotype of Expanded CD8+ T cells

We will assess whether these activated, tumor specific, cytotoxic T cells are clonal. In the post-allogeneic transplant setting, prior studies have shown that response to CD8+ depleted DLI is most successful when there is a high number of exhausted cytotoxic T cells infiltrating the marrow (tumor) prior to therapy.(80) It has also been shown that tumor infiltrating lymphocytes that express the highest frequency T cell receptor beta chain clonotypes are tumor specific.(84) Thus, we will use T cell receptor beta chain complementarity-determining region 3 (TCRB CDR3) region sequencing (ClonoSeq, Adaptive Biotechnologies) to identify clonally-expanded, tumor specific lymphocytes in the marrow prior to HLA-mismatched CD4+ DLI and will confirm that the T lymphocytes that become activated after HLA-

mismatched CD4+ DLI share the same TCRB signatures and, thus, are also tumor specific. TCRB signatures will also be compared between responders and non-responders to support that clinical remissions are due to clonal T cell expansion rather than just a chemotherapy effect.

Aim 3: In patients with MDS/AML who received HLA-mismatched CD4+ DLI therapy, characterize cytokine profiles from the blood associated with severe cytokine release syndrome.

Plasma Cytokine Profile

HLA-mismatched CD4+ DLI is expected to reverse immune exhaustion and stimulate an inflammatory response consistent with cytokine release syndrome (CRS), characterized by fevers and sometimes associated hypotension or hypoxia.(85) Thus, we aim to identify risk factors for severe CRS in patients treated with this novel therapy. Clinically, we will compare baseline variables of patients who experience severe CRS and those who do not, including HLA-mismatched CD4+ DLI dose, degree of HLA mismatch, choice of induction chemotherapy, and disease burden, all factors that are predictive of CRS with other cell therapies.(86-88) In the setting of chimeric antigen receptor T cells (CAR-T) therapy, confirmation that CRS is correlated with high levels of interleukin-6 (IL-6) led to the effective use of IL-6 blockade as CRS treatment.(89-91) We will similarly evaluate cytokine profiles associated with CRS to identify potential targets for anti-CRS therapy. As CRS typically occurs in the first 7 days after other cell therapies, we will collect blood samples prior to therapy and then daily from the day of HLA-mismatched CD4+ DLI infusion through day 7 after infusion. We will test plasma using the Ella multiplex cytokine assay to identify the T cell-derived cytokines (e.g., IFN-gamma, IL-6, IL-17, TNF-alpha) increased at baseline in patients who develop severe CRS and monitor which cytokines significantly increase with onset of CRS. For patients who experience CRS, we will also monitor changes to the cytokine profile after administration of IL-6 blockade (tocilizumab) and correlate temporally with clinical resolution. The association between severe CRS and clinical variables (e.g., DLI dose, degree of HLA mismatch, choice of induction chemotherapy, disease burden) and the change of cytokine profiles after treatment (e.g. IFN-gamma, IL-6, IL-17, TNF-alpha) will be explored by the Satterthwaite t-test. Specifically, cytokine profiles will be measured at baseline, and days 0 through 7. The GEE model may be employed to assess change over time in the difference between two groups. Odds ratios will be estimated along with 95% confidence intervals by using the simple logistic regression or the GEE model. Other statistical considerations apply as discussed in Aim 2. These studies will confirm whether IL-6 is primarily implicated in causing toxicity with HLA-mismatched CD4+ DLI or if other cytokines may be more effective targets for managing this toxicity.

Power calculations

We expect up to 18 subjects will be enrolled in this phase I with cohort expansion study. For Aim 1, up to 18 subjects will have 80% and 98% power to detect an effect size of 0.7 and 1.0 at a two-sided significance level of 5%. The one-sample t-test is used. For Aim 2 and 3, the maximum power is obtained when the sample size of two groups is identical (i.e., 9 subjects per group). For each endpoint of Aim 2 and 3, the study will have 85% power to detect an effect size of 1.5 at a two-sided significance level of 5% when sample size of each group is 9. If the sample

size of one group deviates from 9, the statistical power will decrease, accordingly. Two sample t-test is used for Aim 2 and 3, and no multiplicity adjustment is considered for power analyses.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 DEFINITION OF AE

AE means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related—21 CFR 312.32 (a). An adverse event may include any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite).

8.4.2 DEFINITION OF SAE

An SAE is any sign, symptom or medical condition that emerges during treatment or during a post-treatment follow-up period that (1) was not present at the start of treatment and is not a chronic condition that was part of the patient's medical history, OR (2) was present at the start of treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory criteria:

- is fatal (i.e., results in death from any cause at any time) or life-threatening (i.e., the patient was in the view of the investigator, at immediate risk of death from the reaction as it occurred)
- required or prolonged hospitalization (see exclusions below)
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly or a birth defect
- is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Events not considered to be SAEs are hospitalizations for the:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
- Treatment, which was elective or pre-planned, for a preexisting condition that did not worsen

Serious adverse events occurring in a patient from the first day of treatment and until 4 weeks after the last dose of treatment must be reported. The period after discontinuing study drug may be extended if there is a strong suspicion that the drug has not yet been eliminated.

All serious adverse events must be followed to resolution (≤ 1 or baseline) or until considered stable or irreversible.

8.4.3 CLASSIFICATION OF AN AE

8.4.3.1 SEVERITY OF EVENT

All AEs will be graded using the CTCAE 5.0 criteria.

For AEs not included in the protocol-defined grading system (grade 1 to 4), the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

The grade and severity of the event will be determined using the DCT/NCI Common Terminology Criteria, CTCAE v.5.0. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. Study staff must use one of the CTCAE criteria to define the event. Adverse events not included in the CTCAE v.5.0 should be reported and graded under the "Other" adverse event within the appropriate category and grade 1 to 5 according to the general grade definitions, mild, moderate, severe, life-threatening, fatal or disabling, as provided in the CTCAE.

The event will be determined to be expected or unexpected. The determination of whether an AE is expected is based on agent-specific adverse event information provided in Pharmaceutical Information. Unexpected AEs are those not listed in the agent-specific adverse event information provided in Section 9 Pharmaceutical Information.

Based on this information, a decision will be made whether an adverse event should be reported as an expedited report (Serious Adverse Event 8.4.2) in addition to the routinely reported clinical data. All expedited adverse event reports will be submitted to the Institutional Review Board (IRB) and to the FDA.

Safety will be evaluated based on the incidence, severity, duration, causality, seriousness, and type of adverse events (AEs), and changes in the patient's physical examination, vital signs, and clinical laboratory results. Investigators will use the NCI CTCAE version 5.0 published November 27, 2017. (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf) to assess the severity of adverse events. Observed or volunteered Grade 3-5 adverse events regardless of treatment group or causal relationship to study drug will be recorded on the adverse event pages of the case report form.

There is a known rate of treatment-related mortality associated with induction chemotherapy in this population; in addition, severe infections are common. Therefore, adjudication that an adverse event is possibly related to the exposure, and not the disease or standard treatment (chemotherapy) will be an important role of the Protocol Monitoring Committee.

8.4.3.1.1 LIST AND REPORTING REQUIREMENTS

There are 2 broad categories of reportable adverse events: 1) adverse events that are attributable to use of the device, i.e. the CliniMACS® system with CliniMACS® CD8 Reagent; and 2) adverse events that are not attributable to the use of the device. All reporting of adverse events will be carried out according to current IRB and FDA guidelines.

8.4.3.2 ADVERSE DEVICE EFFECTS

8.4.3.2.1 HYPERSENSITIVITY REACTIONS

Use of the CliniMACS® System enables the infusion of CD8 depleted or selected cells that may contain residual amounts of unbound CliniMACS® CD8 Reagent (murine monoclonal antibody conjugated to an iron dextran moiety). Hypersensitivity reactions resulting from iron dextran therapy have been reported. The most dangerous complication is an anaphylactoid reaction similar to contrast media reactions. The anaphylactoid reaction is usually manifested during the first few minutes of the infusion (often during test dose administration). The most common hypersensitivity reaction occurring in more than 30% of patients given therapeutic levels of iron dextran is the development of arthralgia and fever within 24 to 48 hours after initiation of the intravenous therapy.

8.4.3.2.2 REACTIONS MEDIATED BY HUMAN ANTI-MOUSE ANTIBODIES (HAMA)

The CliniMACS® CD8 Reagent also contains a murine monoclonal antibody. One of the problems limiting the therapeutic use of murine monoclonal antibodies (mAbs) has been the generation of HAMA and subsequent anaphylaxis.(92)The most commonly reported adverse effects have been myalgia, arthralgia, increased peripheral edema and flu-like symptoms, the latter involving fever (70% to 73%), chills (46% to 53%), chest pain and tightness (14%), wheezing (11% to 14%) and vomiting (13% to 32%).(93-95)

8.4.3.3 ADVERSE EVENTS NOT ATTRIBUTABLE TO THE DEVICE

Four categories of device non-attributable adverse events will be monitored and, if necessary, reported: 1) non-hematologic toxicities; 2) GvHD; 3) DLI-induced aplasia; and 4) CRS. Reportable adverse events are defined as non-hematologic toxicities requiring hospitalization, grade 3 or 4 toxicities defined below, and grade II acute GvHD. Adverse events that are not considered serious will be included in the annual report. Serious adverse events include any death within the first 56 days after DLI, grade 2-4 acute GvHD, DLI-induced aplasia, ≥grade 3 CRS, and any adverse event that is deemed to be significant by the PI.

Non-hematologic toxicities will be monitored daily while patient remains admitted (typically until ANC exceeds 500/microliter, which usually occurs between days 21 and 42), then at clinic visits on day 42, day 56, 6 months, and

1 year. Toxicities will be graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

The following is a list of categories that will not be required to be recorded unless the event becomes a grade 4:

- Allergy/Immunology
- Auditory/Hearing
- Cardiovascular (Arrhythmia)
- Cardiovascular (General)
- Coagulation
- Constitutional symptoms
- Dermatology/Skin
- Endocrine
- Hemorrhage
- Hepatic
- Infection/Febrile neutropenia
- Lymphatics
- Metabolic/Laboratory
- Secondary Malignancy
- Sexual/Reproductive Function

A toxicity in the following categories will be captured as an adverse event if it reaches grade 3 or grade 4:

- Gastrointestinal
- Musculoskeletal
- Neurology
- Ocular/Visual
- Pain
- Pulmonary
- Renal/Genitourinary

8.4.3.3.1 GRAFT-VERSUS-HOST DISEASE

GVHD is an unexpected but potential complication of this therapy. Patients will be monitored for GVHD until donor T-cell chimerism decreases to <5% or 1 year from infusion, whichever comes first.

Acute GvHD shall be graded clinically according to the criteria developed by the consensus conference on acute GvHD (see Appendix D).(96) All suspected cases of acute GvHD must be confirmed histologically by biopsy of an affected organ (skin, liver, or gastrointestinal tract). For purposes of reporting, a pathologist will be ultimately responsible for determining whether a patient does or does not have histologic evidence of GvHD. Diarrhea and/or hyperbilirubinemia in a patient with histologically documented skin GvHD may be assumed to be a manifestation of visceral GvHD and will be graded as such. All patients with histologically documented, clinical grade >2 acute GvHD should receive initial treatment with corticosteroids according to institutional preference. If skin GvHD resolves with treatment but suspected visceral GvHD does not, biopsy of the affected organ (liver or gastrointestinal tract) should be obtained to rule out other causes of hyperbilirubinemia and/or diarrhea. Steroid refractory acute GvHD will be treated according to institutional preferences.

The following information shall be collected on all patients with acute GvHD:

- Date of onset (defined as the date of first biopsy confirming GvHD)
- GvHD evaluation form at the time of onset, weekly until GvHD resolves, and/or day 56
- Initial overall clinical grade
- Maximum overall clinical grade
- Date of onset of grade 3-4 acute GvHD, if any
- The occurrence and severity of acute and chronic GvHD after day 56 will be captured at the patient's six-month evaluation.

All instances of grade 2-4 acute GvHD will be captured as serious adverse events.

Chronic GvHD is not an expected complication of the investigational therapy as the DLI should not result in long-term engraftment. Nonetheless, monitoring for chronic GvHD will follow the same procedures as acute GvHD with the following exceptions:

- The severity of GvHD will be determined based on NIH 2014 Consensus Guidelines (see Appendix F).(97)
- Systemic therapy with corticosteroids will be indicated for any patient with moderate or severe chronic GvHD.
- Adjunctive immunosuppressive therapies for severe or steroid refractory chronic GvHD will be determined by the primary provider and institutional preference.

8.4.3.3.2 DONOR LYMPHOCYTE INFUSION-INDUCED APLASIA

DLI-induced aplasia is defined as neutropenia (ANC <500/mL) on day 42 with evidence of donor chimerism (>5%) on the day 42 sample collection. In the case of DLI-induced aplasia diagnosed on day 42, a second chimerism analysis will be sent on day 56 to confirm. All cases of DLI-induced aplasia persisting to day 56 will be reported as serious adverse events.

8.4.3.3.3 CYTOKINE RELEASE SYNDROME

CRS is a recognized complication of the infusion of HLA-mismatched lymphocytes.(51, 98) CRS comprises a constellation of symptoms and signs including fever, hypotension, fluid extravasation leading to edema, ascites and hypoxia, diffuse erythema, diarrhea, and kidney dysfunction. The syndrome is accompanied by elevation in serum markers of inflammation including elevations in acute phase reactants such as interleukin-6, ferritin, and soluble IL-2 receptor. Recent reports demonstrate that CRS can be managed effectively, and symptoms mitigated, by treatment with the anti-IL-6 receptor antibody tocilizumab. (98) Grading of CRS and guidelines for treatment to be used in this study are included in Appendix C.(75) Any instances of CRS grade ≥3 will be reported as serious adverse events.

8.4.3.4 RELATIONSHIP TO STUDY INTERVENTION

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories shown below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have

contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.3.5 EXPECTEDNESS

The PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.4.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

AEs can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a patient.

Grade 3-5 AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured on the appropriate CRF. Information to be collected will include event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

All events beginning with start of study intervention until the first event of either starting allogeneic transplant conditioning, confirmed disease relapse, death, or 1 year from the of the trial cell infusion will be reported. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 AE REPORTING

The PI and/or the research nurse will monitor each patient closely for the development of adverse events and toxicities and record all such events. AEs will be recorded and reported in our annual report. As some toxicities are expected from the chemotherapy, we will compare incidence of toxicities to historical experience when using chemotherapy without the investigational DLI product. The FDA require the timely reporting of adverse events (including toxic deaths).

Each individual sign or symptom must be documented separately. Grade 3-5 adverse events (both expected and unexpected) will be captured on the appropriate study-specific CRFs.

An investigator must verify the attribution of all adverse events. Evaluation of laboratory toxicities may be documented on CRF provided the investigator signs it. However, if an action was conducted due to this abnormality (e.g., red blood cell transfusion due to low hemoglobin) this would be recorded on the AE form also.

AE reporting will begin at the time of cell infusion and continue through 1 year from day 0 or until loss to follow-up, death, disease progression, or proceeding to allogeneic BMT, whichever comes first. Subjects who proceed to allogeneic BMT will come off of the trial and will no longer be followed for AEs.

8.4.6 SAE REPORTING

The study clinician will immediately report to the FDA any SAEs, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are SAEs (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the DCC/study sponsor and should be provided as soon as possible.

It is the responsibility of the PI and the research team to ensure that SAEs are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and IRB policy.

All adverse experience reports must include the patient number, age, sex, weight, severity of reaction (mild, moderate, severe), relationship to study drug (probably related, unknown relationship, definitely not related), date and time of administration of test medications, all concomitant medications, and medical treatment provided. The PI is responsible for evaluating all AEs to determine whether criteria for "serious" as defined above are present. Adverse drug reactions that are serious, unlisted/unexpected, and at least possibly associated to the drug, and that have not previously been reported in the IB, or reference safety information document should be reported promptly to the FDA in writing by each investigator/physician engaged in clinical research. A clear description of

the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

8.4.7 REPORTING EVENTS TO PARTICIPANTS

As these medications and procedures are part of a Phase 1/2 protocol, their side effects have not been evaluated or approved for safety by the FDA. Although it is anticipated that this protocol is relatively safe, fatal complications are possible. The major hazards are infection and disease progression. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. The major discomforts are nausea, mucositis, anorexia, diarrhea, fever and malaise. Physicians have an ethical responsibility to inform patients about AEs and SAEs.

Patients will be addressed by a nurse or physician to disclose AEs and SAEs. These individuals will explain the AE/SAE in an honest, open, and direct manner in a private setting. The nurse or physician will be given a detailed description of the event, incident, experience or outcome, as well as an explanation as to why it is considered an AE/SAE. They will be informed of any changes to protocol or corrective actions that will result from the AE/SAE. The physician will continue to monitor the AE/SAE in the most appropriate manner for the situation and will, if necessary, withdraw the patient from the study.

Other patients enrolled in the study will be informed of changes to protocol that are resultant of another participant's AE/SAE. They will not be given any personal details of the patient of their AE/SAE in order to protect patient privacy.

Patients will be encouraged to communicate any incidental side effects or information discovered during study procedures. Patients will be holistically monitored for signs of any unintentional or incidental effects of the study. Physicians will communicate any incidental findings that may affect the patient or the patients' desire to participate in the study.

8.4.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.4.9 REPORTING OF PREGNANCY

Pregnancy, although not itself an SAE, should also be reported on an SAE form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

8.5 UNANTICIPATED PROBLEMS

8.5.1 DEFINITION OF UP

The OHRP considers UPs involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.5.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report UPs to the reviewing IRB and to the DCC/lead PI. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB, depending on their policy, and to the DCC/study sponsor within 7 working days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 7 working days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the OHRP within 7 working days of the IRB's receipt of the report of the problem from the investigator.

An investigator shall submit to the sponsor and to the reviewing IRB a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 7 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1)), A sponsor who conducts an evaluation of an unanticipated adverse device effect under 812.46(b) shall report the results of such evaluation to the FDA and to all reviewing IRB's and participating investigators within 7 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1)).

8.5.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

As these medications and procedures are part of a Phase 1/2 protocol, they may cause wholly unknown and unexpected side effects. Unexpected problems will be reported to patients as soon as possible.

A nurse or physician will be given a detailed description of the event, incident, experience or outcome, as well as an explanation as to why it is considered a UP. They will be informed of any changes to protocol or corrective actions that will result from the UP.

Other patients enrolled in the study will be informed of changes to protocol that are resultant of another participant's UP. They will not be given any personal details of the patient of their UP in order to protect patient privacy.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

We hypothesize that a source of CD8+ depleted, non-engrafting, HLA-mismatched, unrelated donor lymphocyte infusion (NE-DLI) would be capable of reversing immune tolerance and eliciting a significant antitumor response in patients with MDS and secondary AML, without causing GVHD.

Primary Endpoint:

- Determine the maximum tolerated dose (MTD) of CD8$^-$depleted cells from HLA-mismatched unrelated donors infused after induction chemotherapy for patients with advanced MDS after HMA failure or untreated sAML

Secondary Endpoints:

To determine:

- Overall response rate according to the International Working Group (IWG) (see Appendix B).
- Overall survival.
- Progression-free survival.
- Hematologic response/improvement by IWG 2006 criteria for MDS patients and the IWG 2003 criteria for AML (1, 2).
- Occurrence of adverse events.
- Unexpected transplant complications (eg, graft-versus-host disease [GvHD]).

9.2 SAMPLE SIZE DETERMINATION

See Sections 9.4.2 and 9.4.4

9.3 POPULATIONS FOR ANALYSES

Not Applicable

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

This is a phase 1b/2 study with dose escalation for determination of the MTD/RP2D of NE-DLI via a traditional 3+3 dose escalation schema[a] followed by an expansion cohort for assessing safety profile and preliminary estimation of the overall response rate (ORR) (complete response and partial response based on International Working Group definition). Eligible patients will be treated with NE-DLI in the pre-transplantation setting.

9.4.1.1 ANALYSIS PLAN

[a] See Appendix F

Toxicity events for NE-DLI will be descriptively summarized at each dose level. The response rates, including ORR and hematologic response by IWG 2006 criteria will be estimated via an exact binomial test with 95% confidence interval in combining patients treated at the RP2D dose level and the expansion cohort patients. Overall survival, defined from date of treatment to date of death or censored at last follow-up date, will be estimated via Kaplan-Meier method (combining patients treated at the RP2D dose level and expansion cohort patients). Other outcomes such as independence from red blood cell transfusion or platelet transfusion for 56 consecutive days or more, occurrence of AE, unexpected transplant complications will be descriptively reported via summary statistics.

9.4.2 ANALYSIS OF THE PRIMARY ENDPOINT(S)

For the dose escalation portion, a minimum of two subjects and a maximum of 18 subjects will be enrolled. There will be three dose levels in the dose escalation portion. MTD is defined as the highest dose level with the probability of DLT less than 33% (DLT events are defined in Section 6). The DLT observation window will be until confirmation that the donor cells have been rejected (ANC $\geq$ 500/microL AND confirmation of either bone marrow donor chimerism <5% or donor blood CD3+ chimerism <5%) OR within 56 days following NE-DLI, whichever occurs first. Per the traditional 3+3 schema, up to three patients may be enrolled concurrently and treated at the same dose level. The first three patients will be treated at dose level 1. For each dose level, if none of three patients experiences a DLT, we will escalate to the next dose level until reaching dose level 3. For each dose level, if one of the three patients experiences a DLT, we will enroll three additional patients and treat at the same dose level. In this case, if none of these additional three patients experiences a DLT, we will escalate the dosage to next level until reaching dose level 3. For each dose level, if two or more patients among six enrolled patients experience a DLT, or two or more patients in the three patient cohort experience DLT, the dosage level exceeds the MTD and the previous dose level will be declared as the MTD. If the first dose level exceeds the MTD, the study will be suspended. If no subjects experience a DLT at any dose level, the MTD will be level 3 (5×10^7 CD4+ T cells/kg). Similarly, if exactly one of six patients enrolled at dose level 3 experiences a DLT, then the MTD will be dose level 3. All patients in a dose level must be observed through the DLT window prior to enrolling patients at a higher dose level.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The RP2D is defined as the dose level selected to be evaluated in the expansion cohort or future phase II studies and will be equivalent to the MTD determined in the Phase 1b portion of the study. Once we determine the RP2D level, we will enroll 10 additional eligible patients and treat with the RP2D level. The goal for this expansion cohort is to evaluate the safety and tolerability for patients who will be treated with NE-DLI in the RP2D dose level, and to evaluate the preliminary efficacy estimate for the ORR. Including the dose escalation portion in which 3 or 6 enrolled patients will have been treated at the RP2D level, a total of at least 13 patients will be treated at the RP2D level. Based on previous data,(99) the complete response rate using microtransplantation was in the range of 36%-52% for MDS or AML from antecedent MDS patients. Hence, for the phase 2 portion, an ORR of 45% is considered clinically meaningful. With a sample size of 13 patients, if our ORR is 45%, the 95% CI (exact binomial) will be (18-74%); if the ORR is 50%, the 95% CI will be (22-78%). The response rates, including ORR and hematologic response by IWG 2006 criteria will be estimated via an exact binomial test with 95% confidence interval in combining patients treated at the RP2D dose level and the expansion cohort patients.

9.4.4 SAFETY ANALYSES

We will monitor the toxicity events for the expansion cohort for every 2 patients after the first 3 patients. We will apply a Bayesian toxicity monitoring rule: if the posterior probability of experiencing DLT exceeding 0.25 is greater than 65%, we will hold the enrollment for reviewing the MTD. The prior distribution for this monitoring rule is a beta,(1,5) which translates to a 90% probability of DLT being between 1% and 45.1%. The stopping rules for toxicity monitoring and operating characteristics for expansion cohort are listed in the following:

Table 1: Early stopping rule for monitoring toxicity

Number of Patients	3	4-6	7-10
Number of Patients with DLT resulting in escalation suspension	2	3	4

Table 2: **Operating Characteristics of stopping rule for toxicity, based on 50,000 simulations:**

True Toxicity Rate	Probability of declaring treatment too toxic	Average sample size
15%	7.1%	9.8
20%	15.3%	9.5
25%	26.5%	9.2
30%	39.6%	8.7
35%	52.9%	8.2
40%	65.4%	7.7
45%	76%	7.2

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Not applicable

9.4.6 PLANNED INTERIM ANALYSES

See section 9.4.2. If the first dose level exceeds the MTD, the study will be suspended.

9.4.7 SUBGROUP ANALYSES

Not applicable

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Not applicable

9.4.9 EXPLORATORY ANALYSES

Toxicity events for NE-DLI will be descriptively summarized at each dose level. Overall survival, defined from date of treatment to date of death or censored at last follow-up date, will be estimated via Kaplan-Meier method (combining patients treated at the RP2D dose level and expansion cohort patients). Other outcomes such as independence from red blood cell transfusion or platelet transfusion for 56 consecutive days or more, occurrence of AE, unexpected transplant complications will be descriptively reported via summary statistics.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

A study investigator, study research nurse, or clinical trial coordinator working on this study will obtain written informed consent. An explanation of the nature of study, its purpose, procedures involved, expected duration, potential risks and benefits will be provided to each participant by the investigator or the research nurse. Each participant will be informed that participation in the study is voluntary and that he may withdraw from the study at any time, and that withdrawal of consent will not affect his subsequent medical treatment. Participants will be allowed time needed to make an informed decision. Participants will be encouraged to ask questions about the study and the consent before signing the consent form. Consent forms will be filed with the Clinical Research Office and copies stored securely with the study coordinator. No patient will enter the study before his informed consent has been obtained.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved and the participant will be asked to read and review the document.

The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants.

Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study.

Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their

records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the IND sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

10.1.3 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the Moffitt Cancer Center. After the study is completed, the de-identified, archived data will be transmitted to and stored at the Moffitt Cancer Center, for use by other researchers including those outside of the study. Permission to use this data will be included in the informed consent.

These samples could be used to research the causes of myelodysplastic syndrome and/or acute myeloid leukemia, its complications and other conditions for which individuals with myelodysplastic syndromes/acute myeloid leukemia are at increased risk, and to improve treatment. The Moffitt Cancer Center will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the Moffitt Cancer Center.

10.1.4 KEY ROLES AND STUDY GOVERNANCE

PI
Hany Elmariah, MD, MS *Moffitt Cancer Center* *12902 USF Magnolia Drive* *813-745-8248* *Hany.elmariah@moffitt.org*

10.1.5 SAFETY OVERSIGHT

SAEs from this protocol will be reported concurrently to the IRB and the study sponsor. The Protocol Monitoring Committee (PMC) will review these SAEs in accordance with the protocol-specific DSMP. The data and safety plan will define dose limiting toxicities, rules for escalation of dose, and criteria for stopping the trial and defining the MTD according to rules set forth by this protocol. This trial will be continuously monitored by the PI and the research team and reviewed at bi-weekly Immunotherapy/Cell Therapy Research Group meetings. Safety and monitoring reports will be submitted to the PMC after completing each odd numbered dose level (ie, 1, 3, 5, etc.) or more frequently if requested by the PMC. A final safety and monitoring report will be submitted to the PMC within three

months of defining the MTD. This protocol will be subject to periodic internal audits based on risk or as recommended by the PMC.

10.1.6 CLINICAL MONITORING

MCC's Internal Monitors will periodically monitor regulatory documents and CRFs according to the protocol specific CMP. Monitoring will include review of data for accuracy, completeness, and source verification, reporting of SAEs, and adherence to the protocol, GCP guidelines, and applicable regulatory requirements.

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.8 DATA HANDLING AND RECORD KEEPING

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data will be captured in OnCore and/or Moffitt's electronic CTMS. For each subject enrolled, the electronic CRF must be completed by the assigned data manager or other authorized study staff. Clinical data will be entered directly from the source documents. Any paper forms should be typed or filled out indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. This also applies to records for those subjects who fail to complete the study. If a subject stops dosing or terminates from the study, the dates and reasons must be noted on the CRF. If a subject terminates from the study because of a DLT, thorough efforts should be made to clearly document the outcome.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

10.1.8.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 10 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 10 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when there is no longer a need for these documents to be retained. Permission must be acquired from the State of Florida for document destruction after the 10-year minimum record-retention period described above has elapsed.

10.1.9 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 QA and QC, Section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP. Deviations must be entered into the CTMS.

10.1.10 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the Food and Drug Administration (FDA) publication and data sharing policies and regulations for FDA funded research:

FDA will ensure grantee and contractor compliance with planned publication access and data management requirements by requiring, as a term and condition of the grant or contract award, periodic reporting to contracting officer representatives and program officers as a part of regular grants and contract management. Extramural researchers will periodically report

• all published articles resulting from research funding and the unique FDA manuscript repository identifier associated with the article; and

• the status of data collection and preservation, including deviation from the approved data management plan.

Failure to comply with the publication access and data management requirements—including the periodic reporting requirements—may serve as grounds to terminate the contract or cancel the grant. This decision rests with the contracting or grants management officer and will be made only after a careful review of all relevant facts and circumstances, and with appropriate consultation with the program office administering the grant or contract.

Prior to funding new grant and contract awards or continuing non-competing continuation awards, FDA will confirm awardees are in compliance with the FDA Public Access Policy. FDA will not award a grant or contract until an awardee has come into compliance with the policy.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ABBREVIATIONS

MDS	Myelodysplastic syndrome
HMA	Hypomethylating Agent
sAML	Secondary acute myeloid leukemia
MTD	Maximum Tolerated Dose
IWG	International Working Group
GVHD	Graft-versus-host disease
ANC	Absolute neutrophil count
CBC	Complete blood count
CMP	Comprehensive metabolic panel
CMV	Cytomegalovirus
CXR	Chest x-ray
Diff	Differential blood count
Echo	Echocardiogram
EKG	Electrocardiogram
MUGA	Multigated acquisition (cardiac)
PCR	Polymerase chain reaction
Allo	Allogeneic
BMT	Bone marrow transplant
PD1	programmed cell death protein 1
PDL1	PD1 ligand
CTLA4	cytotoxic T lymphocyte associated protein 4
DLI	Donor lymphocyte infusion
NE-DLI	Nonengrafting DLI (CD8 depleted DLI)
AHD	Antecedent hematologic disorder
WHO	World Health Organization
RP2D	Recommended phase 2 dose
ATRA	All trans-retinoic acid
ECOG	Eastern Cooperative Oncology Group
CrCl	Creatinine clearance
IND	Investigational New Drug Application
IRB	Investigational Review Board
LVEF	Left ventricular ejection fraction
MCC	Moffitt Cancer Center
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NIH	National Institutes of Health
NIH IC	NIH Institute & Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOC	System Organ Class
SOP	Standard Operating Procedure
IBW	Ideal Body Weight

CONSORT	Consolidated Standards of Reporting Trials
DLT	Dose Limiting Toxicity
ORR	Overall Response Rate
CRS	Cytokine Release Syndrome
CRF	Case Report Forms
FACT	Foundation for the Accreditation of Cellular Therapy
AE	Adverse Event
CTCAE	Common Terminology Criteria for Adverse Events
NCI	National Cancer Institute
HAMA	Human anti-mouse antibodies
mAbs	Monoclonal antibodies
UP	Unanticipated Problems

10.3 PROTOCOL AMENDMENT HISTORY

Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB before implementation.

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
2.0	1/25/2019	Daley Drucker formatted into MCC Template; minor editing and changes.	All Moffitt Cancer Center Clinical Trial Protocols must be in the MCC template.
2.1	2/8/2019	Dr. Elmariah added additional information and reviewed Version 2.0 changes.	Completed the template and reviewed for clarity.
2.2	2/11/2019	Daley Drucker addressed Dr. Elmariah's questions and provided secondary minor edits and reformatting.	Answered Dr. Elmariah's questions, provided consistent formatting.
3.0	2/12/2019	Dr. Elmariah edits	Approval of comments by Daley Drucker
3.1	2/14/2019	Daley Drucker review and final edits.	Finalizing format.
3.2	2/14/2019	Paul Fletcher reviewed Drucker's formatting before resubmission to PI.	Finalize formatting

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12 APPENDIX

12.1 APPENDIX A: INTERNATIONAL PROGNOSTIC SCORING SYSTEM- REVISED OF MYELODYSPLASTIC SYNDROME(58)

Table 1: Prognostic score variables

Prognostic Variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very Good		Good		Intermediate	Poor	Very Poor
BM Blast %	<=2		>2-<5%		5-10%	>10%	
Hemoglobin	=>10		8-<10	<8			
Platelets	=>100	50-<100	<50				
ANC	=>0.8	<0.8					

Table 2: Cytogenetic Risk Groups

MDS Cytogenetic Scoring System

Prognostic subgroups, % of patients	**Cytogenetic abnormalities**
Very good (4%[*]–/3%[†]–)	−Y, del(11q)
Good (72%[*]–/66%[†]–)	Normal, del(5q), del(12p), del(20q), double including del(5q)
Intermediate (13%[*]–/19%[†]–)	del(7q), +8, +19, i(17q), any other single or double independent clones
Poor (4%[*]–/5%[†]–)	−7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: 3 abnormalities
Very poor (7%[*]–/7%[†]–)	Complex: > 3 abnormalities

Table 3: Risk Categories

RISK CATEGORY	RISK SCORE
Very Low	≤1.5
Low	>1.5-3
Intermediate	>3-4.5
High	>4.5-6
Very High	>6

12.2 APPENDIX B: RESPONSE CRITERIA ACCORDING TO THE INTERNATIONAL WORKING GROUP(1)

Category	Response criteria (responses must last at least 4 wk)
Complete remission	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines*
	Persistent dysplasia will be noted*†
	Peripheral blood‡
	Hgb ≥ 11 g/dL
	Platelets ≥ 100 × 10^9/L
	Neutrophils ≥ 1.0 × 10^9/L†
	Blasts 0%
Partial remission	All CR criteria if abnormal before treatment except:
	Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%
	Cellularity and morphology not relevant
Marrow CR†	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment†
	Peripheral blood: if HI responses, they will be noted in addition to marrow CR†
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 wks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following:
	Return to pretreatment bone marrow blast percentage
	Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets
	Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete
	Disappearance of the chromosomal abnormality without appearance of new ones
	Partial
	At least 50% reduction of the chromosomal abnormality
Disease progression	For patients with:
	Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts
	5%-10% blasts: ≥ 50% increase to > 10% blasts
	10%-20% blasts: ≥ 50% increase to > 20% blasts
	20%-30% blasts: ≥ 50% increase to > 30% blasts
	Any of the following:
	At least 50% decrement from maximum remission/response in granulocytes or platelets
	Reduction in Hgb by ≥ 2 g/dL
	Transfusion dependence
Survival	Endpoints:
	Overall: death from any cause
	Event free: failure or death from any cause
	PFS: disease progression or death from MDS
	DFS: time to relapse
	Cause-specific death: death related to MDS

Deletions to IWG response criteria are not shown.

To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.

MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-American-British; AML, acute myeloid leukemia; PFS, progression-free survival; DFS, disease-free survival.

*Dysplastic changes should consider the normal range of dysplastic changes (modification).[41]

†Modification to IWG response criteria.

‡In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 4-week period. Such patients can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.

12.3 APPENDIX C: EVALUATION AND MANAGEMENT OF CYTOKINE RELEASE SYNDROME(75)

Grading of Cytokine Release Syndrome
(Assess daily and any time there is a change in patient status)

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temperature ≥ 38°C	Temperature ≥ 38°C	Temperature ≥ 38°C	Temperature ≥ 38°C
With either:				
Hypotension	None	Not requiring vasopressors	Requiring one vasopressor (with or without vasopressin)	Requiring multiple vasopressors (excluding vasopressin)
❖ And/or				
Hypoxia	None	Requiring low-flow nasal cannula	Requiring high-flow nasal cannula, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg: CPAP, BiPAP, intubation and mechanical ventilation)

Adapted from Lee DW, et al. Biol Blood Marrow Transplant. 2018 Dec 25. [Epub ahead of print]

- ❖ CRS grade is determined by the most severe event: hypotension or hypoxia not attributable to any other cause
- Organ toxicities associated with CRS may be graded according to CTCAE v5.0, but do not influence CRS grading

Terms	Definitions
Fever	• Temperature ≥ 38°C not attributable to any other cause. • In patients who have CRS then receive antipyretics or anti-cytokine therapy such as tocilizumab/steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.
Low-flow nasal cannula	• Oxygen delivered at ≤ 6 liters/minute. • Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics.
High-flow nasal cannula	• Oxygen delivered at >6 liters/minute.

Management of Cytokine Release Syndrome

CRS Grade	Sign or Symptom	Management
Grade 1	**Fever**	• Symptomatic management of constitutional symptoms and organ toxicities • Acetaminophen and hypothermia blanket as needed for fever • Assess for infection, empiric broad spectrum antibiotics • IV fluids as needed • Consider tocilizumab for persistent fever lasting >3 days in patients with significant comorbidities or if patient is deteriorating
Grade 2 All Grade 2 Cardiac telemetry and pulse oximetry, consider ECHO	**Hypotension** *Not requiring vasopressors*	• IV fluid bolus of NS 500-1000 mL • If patient requires multiple fluids boluses assess fluid balance and consider tocilizumab. • For hypotension refractory to fluid boluses: tocilizumab 8 mg/kg IV. o For high risk patients *consider* tocilizumab + dexamethasone 10 mg IV x one • If no response, consider redosing tocilizumab 8 mg/kg IV (may be repeated every 8 h for up to 3 doses in a 24 h period) • If hypotension persists after fluids boluses and 1-2 doses of tocilizumab, or if patient is not improving or deteriorating: o Consider dexamethasone 10 mg IV every 6 hours. o Manage as grade 3 CRS (start vasopressors, transfer to ICU and obtain ECHO) • Symptomatic management of constitutional symptoms and organ toxicities
	Hypoxia *(Low-flow nasal cannula → O_2 delivered at ≤ 6 L/min)*	• Supplemental oxygen as needed • Tocilizumab 8mg/kg IV o For high risk patients *consider* tocilizumab + dexamethasone 10 mg IV x one • If hypoxia persists after above interventions; however oxygen requirement is stable, continue close monitoring. • Tocilizumab may be repeated every 8 h for up to 3 doses in a 24 h period • If oxygen requirement increases or patient deteriorates: o Consider dexamethasone 10 mg IV every 6 hours o Manage as Grade 3 CRS • Symptomatic management of constitutional symptoms and organ toxicities
Grade 3	**Hypotension** *Requiring one vasopressor +/- vasopressin*	• IV fluid boluses as needed, vasopressors as needed • Transfer to ICU, obtain ECHO if not performed already • Tocilizumab if not administered previously • Start dexamethasone 10 mg IV every 6 hours if not started previously. Alternatively methylprednisolone 1 mg/kg IV every 12 hours may be used. • Symptomatic management of constitutional symptoms and organ toxicities

	Hypoxia *(High-flow nasal cannula → O_2 delivered at ≥6 L/min)*	• Supplemental oxygen as needed • Tocilizumab if not administered previously • Start dexamethasone 10 mg IV every 6 hours if not started previously. Alternatively methylprednisolone 1 mg/kg IV every 12 hours may be used. • Symptomatic management of constitutional symptoms and organ toxicities
Grade 4	**Hypotension** *Requiring multiple vasopressors*	• Vasopressors, tocilizumab and ECHO as above • Consider changing corticosteroids to high dose methylprednisolone 1000mg/day IV. • Symptomatic management of constitutional symptoms and organ toxicities
	Hypoxia *Requiring positive pressure*	• Supplemental oxygen requiring positive pressure ventilations: (CPAP, BiPAP, intubation and mechanical ventilation) • Tocilizumab • Consider changing corticosteroids to high dose methylprednisolone 1000 mg/day IV. • Symptomatic management of constitutional symptoms and organ toxicities

Adapted from Neelapu S, et al. Nat Rev Clin Oncol. 2018;15:47-62, Lee DW, et al. Biol Blood Marrow Transplant. 2018 Dec 25. [Epub ahead of print]

High risk for CRS: bulky disease, co-morbidities, early onset of CRS within 3 days of infusion

Table 3. Dosing

Drug	Dose	Notes
Tocilizumab[1]	8 mg/kg IV over 60 minutes (total volume 100 mL) Up to three doses in 24 hours, up to 4 doses total. Doses a minimum of 8 hours apart Maximum dose of 800 mg	First line treatment for CRS May be used for treatment of ICANS if associated with concurrent grade 2 CRS
Dexamethasone	10 mg IV every 6 hours	Continue until improvement to grade 1 or less and then consider taper
Methylprednisolone	1 mg/kg every 12 hours	Alternative to dexamethasone
High dose methylprednisolone	500 mg IV every 12 hours (1000 mg/day) for 3 days followed by -250 mg IV every 12 hours for 2 days, -125 mg IV every 12 hours for 2 days, -60 mg IV every 12 hours	Taper after improvement to grade 1 CRS or ICANS

12.4 APPENDIX D: CONSENSUS CONFERENCE CLINICAL GRADING OF ACUTE GVHD(96)

Clinical Staging

Stage	Skin	Liver: Total Bilirubin	Intestinal Tract: Diarrhea
0	No rash	<2.0 mg/dL	≤500 ml/day
1	<25% of skin surface	2.0-3.0	500-1000 ml/day
2	25-50%	3.1-6.0	1001-1500 ml/day
3	Erythroderma	6.1-15.0	>1500 ml/day
4	Erythroderma with bullae and desquamation	>15.0	Severe abdominal pain with or without ileus

Clinical Grading

Grade	Skin*	Liver	GI
I	1-2	0	0
II	3	1	1
III	-	2-3	2-4
IV	4	4	-

*Each column identifies minimum stage for organ grade.

12.5 APPENDIX E: GRADING OF CHRONIC GVHD(97)

	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, capabl of self-care, >50% of waking hours ou 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† ☐ **SCORE % BSA** *GVHD features to be scored by BSA:* **Check all that apply:** Maculopapular rash/erythema; Lichen planus-like features; Sclerotic features; Papulosquamous lesions or ichthyosis; Keratosis pilaris-like GVHD	No BSA involved	1-18% BSA	19-50% BSA	>50% BSA
SKIN FEATURES SCORE:	No sclerotic features		**S**uperficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** Deep sclerotic features; "Hidebound" (unable to pinch); Impaired mobility; Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** Hyperpigmentation; Hypopigmentation; Poikiloderma; Severe or generalized pruritus; Hair involvement; Nail involvement; *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
MOUTH *Lichen planus-like features present:* **Yes** **No**	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
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* **Yes** **No** **Not examined**	No symptoms	Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GI Tract ***Check all that apply:*** Esophageal web/ proximal stricture or ring Dysphagia Anorexia Nausea Vomiting Diarrhea Weight loss ≥5%* Failure to thrive	No symptoms	Symptoms without significant weight loss* (<5%)	Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
LIVER	Normal total bilirubin and ALT or AP < 3 x ULN	Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	Elevated total bilirubin > 3 mg/dL
Abnormality present but explained entirely by non-GVHD documented cause (specify):				
LUNGS**				
Symptom score:	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)
Lung score: **%** FEV1 ☐	FEV1≥80%	FEV1 60-79%	FEV1 40-59%	FEV1 ≤39%
Pulmonary function tests Not performed				
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

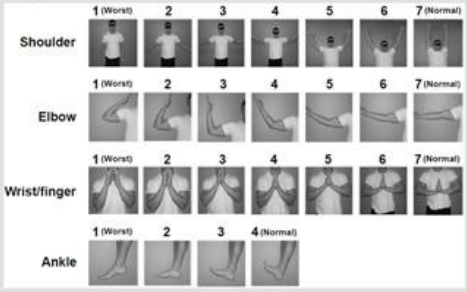
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	No symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs **OR** 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.)
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) Not examined *Currently sexually active* Yes No	No signs	Mild signs‡ and females with or without discomfort on exam	Moderate signs‡ and may have symptoms with discomfort on exam	Severe signs‡ with or without symptoms
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

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

Ascites (serositis)___	Myasthenia Gravis___	
Pericardial Effusion___	Peripheral Neuropathy___	Eosinophilia > 500/µl___
Pleural Effusion(s)___	Polymyositis___	Platelets <100,000/µl ___
Nephrotic syndrome___	Weight loss>5%* without GI symptoms ___	Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ❑ **No GVHD** ❑ **Mild** ❑ **Moderate** ❑ **Severe**

Photographic Range of Motion (P-ROM)



† Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring.

* Weight loss within 3 months.

**Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

Abbreviations: ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); ULN (normal upper limit).

‡ To be completed by specialist or trained medical providers (see Supplemental Figure).

12.6 APPENDIX F: CRITERIA FOR DOSE ESCALATION

Dose escalation will occur if 0 out of the first 3 patients experience DLTs. Rules for dose escalation are as follows:

0/3 DLTs: Escalate the dose (dose levels 1-2), or halt dose escalation (dose level 3).

1/3 DLTs: Study an additional 3 patients. If an additional patient experiences a DLT, halt dose escalation. If no other patient experiences DLT (that is if 1/6 patients on dose level experiences DLT), escalate the dose (dose levels 1-2) or halt dose escalation (dose level 3).

2/3 DLTs: Halt dose escalation.

The decision to escalate the dose of donor peripheral blood cells and to enroll patients to the higher dose level may not be made until one of the following has occurred:

1. The first 3 patients have been fully evaluated for DLTs and none has experienced a DLT.
2. Six patients have been fully evaluated and only 1 has experienced a DLT.

The DLT observation window continues until the first of the following:

1. ANC $\geq$ 500/microL AND confirmation the DLI has been rejected (either bone marrow donor chimerism <5% or donor blood CD3+ chimerism <5%)
2. Within 56 days following NE-DLI

If neither of these conditions has been met, patients will continue to be accrued to the current dose level. In the event that 0/3 patients experience DLT, accrual to the next higher dose level may proceed even if additional patients have been treated at the lower dose level and are not yet evaluable. However, if any of those patients experience DLT, accrual to the higher dose level will be suspended until a total of 6 patients have been treated and evaluated at the lower dose level. Further accrual to the higher dose level will not resume until the DLT incidence in the lower dose level is documented to be no greater than 1/6 patients. Accrual to the higher dose level will be stopped if the incidence of DLTs in the previous level exceeds 33% (for instance, if none of the first 3 patients experiences DLT, but the next 2, who were accrued during the observation period for the first 3 patients, do).

DLTs are defined as any of the following that is at least possibly attributable to the investigational treatment:

1. Grade 2-4 infusion toxicity
2. Grade 4 organ toxicity (as defined by NCI CTCAE v 5.0), not preexisting, not due to the primary malignancy, and not due to chemotherapy or infection
3. Grade 3 organ toxicity that does not resolve within 14 days and which is not preexisting, not due to the primary malignancy, and not due to chemotherapy or infection
4. Grade 2-4 acute GvHD
5. Delayed neutrophil recovery: ANC>500/μl does not occur within 56 days AND measurable donor chimerism is >5%
6. Grade 3-4 CRS; see Appendix for grading scheme)
7. Any Grade 5 reaction other than progressive disease.

If 2 or more of the first 6 patients on any dose level experience DLTs, accrual to the trial will be suspended and the sponsor of the IDE (the PI) will initiate a formal consultation with the FDA in order to determine whether the

observed toxicities are or are not attributable to the device. Subsequent accrual to the trial may resume only with permission from the FDA. Any questions or concerns as to the relationship of grade > 3 toxicities to use of the investigational device will be discussed with the appropriate FDA Clinical Review Staff.