Allogeneic Hematopoietic Stem Cell Transplantation as Initial Salvage Therapy for Patients with Primary Induction Failure Acute Myeloid Leukemia Refractory to High-Dose Cytarabine-Based Induction Chemotherapy

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Principal Investigator: Stefan O. Ciurea, MD
The University of Texas MD Anderson Cancer Center
Stem Cell Transplantation and Cellular Therapy Department
1515 Holcombe Blvd, Unit 0432
Houston, TX 77030
Telephone: 713.745.0146
Fax: 713.794.4747
sciurea@mdanderson.org

Co-Chairs: Elias Jabbour, MD[1]
Naval Daver, MD[1]

Collaborators: Michael Andreeff, MD, PhD[1]
Richard E. Champlin, MD[2]
Partow Kebriaei, MD[2]
Marina Konopleva, MD, PhD[1]
Steven M. Kornblau, MD[1]
Victor Mulanovich, MD[4]
Betul Oran, MD[2]
Uday R. Popat, MD[2]
Farhad Ravandi-Kashani, MD[1]
Rebecca Tidwell, MS[3]

[1] The University of Texas MD Anderson Cancer Center, Leukemia Department
[2] The University of Texas MD Anderson Cancer Center, Stem Cell Transplantation and Cellular Therapy Department
[3] The University of Texas MD Anderson Cancer Center, Biostatistics Department
[4] The University of Texas MD Anderson Cancer Center, Infectious Disease Department

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Protocol Body

1.0 Objectives

Primary Objectives:
1. To determine the safety and feasibility of allogeneic hematopoietic stem cell transplantation (AHSCT) as initial salvage treatment for patients with primary induction failure (PIF) acute myeloid leukemia (AML).

2. To determine efficacy of AHSCT following decitabine, clofarabine, idarubcine, ~~and~~cytarabine (DCIA) and venetoclax salvage chemotherapy evaluated by overall response rate (RR), defined as complete response (CR) or CR without platelet recovery (CRp) or CR with insufficient hematological recovery (CRi).

Secondary Objectives:
1. To determine the percentage of patients with PIF AML eligible for AHSCT after up to 2 courses of induction chemotherapy.

2. To determine the early treatment-related mortality (TRM) (within first 4 weeks of first salvage chemotherapy regimen with DCIA and day 100 TRM after AHSCT.

3. To determine the efficacy DCIA regimen as salvage chemotherapy for patients with PIF AML (% of patients who achieve </=5% bone marrow blasts prior to AHSCT.

4. To determine the TRM at 1 year, relapse rate (RR), overall survival (OS) and event-free survival (EFS) for patients with PIF AML treated with DCIA followed by early AHSCT.

2.0 Background and Rationale

Acute myeloid leukemia
AML is a malignancy of immature granulocytes and/or monocytes. This disease is characterized by accumulation of leukemic blastocytes and blockade of normal bone marrow production resulting in thrombocytopenia, anemia, and neutropenia. There are approximately 13,000 new cases of AML per year in the United States, with an estimated 10,000 deaths occurring in the same time period.[1]. Almost all newly diagnosed AML cases will be in adults.[2] Standard treatment for AML includes systemic combination chemotherapy to control bone marrow and systemic disease. Treatment is generally divided into an induction phase, to attain remission, and a maintenance phase.[2] Approximately 60% to 70% of adults with AML can be expected to attain complete remission status following appropriate induction therapy. Remission rates in adult AML are inversely related to age, with an expected remission rate of > 65% for those younger than 60 years. Increased morbidity and mortality during induction appear also to be directly related to age.[3-5]

AML Refractory to HDAC-based induction chemotherapy
A high-dose cytosine arabinose (HDAC) containing regimen has been standard induction therapy for all AML patients at MD Anderson Cancer Center. The outcome of AML patients who are refractory to HDAC or other AML induction regimens is dismal, with low response rates to salvage chemotherapy and poor long-term survival. [6-8] Allogeneic hematopoietic stem cell

transplantation (AHSCT) is the only salvage option with true curative potential in this scenario.[9, 10] The concept of performing AHSCT in patients who are not in complete response (CR) is not totally novel. Several studies have reported outcomes in patients with primary refractory AML who proceed to AHSCT while not in CR. It must be noted that many of these studies used standard induction regimens consisting of cytarabine (100- 200 mg/m for 7 days) in combination with an anthracycline, administered intravenously for 3 days.

Approximately, 20-40% of adults with AML fail to achieve CR with AML induction regimens (defined as 1 gm/m^2 or higher cytarabine per dose), and are deemed PIF. We have previously reported a dismal response rate (11%) and poor median OS (2.9 months) for patients with AML who are PIF and went on to receive salvage with chemotherapy alone.[7] Conversely, PIF patients at MD Anderson Cancer Center who received initial salvage with AHSCT had significantly superior outcomes including a response rate of 82% and a median OS of 14.9 months (P<0.001) (Jabbour E, et al. accepted for publication). The 3-year OS was 39% for patients undergoing initial AHSCT versus 2% for patients undergoing salvage chemotherapy (P<0.001), indicating the potential for cure in a significant proportion of PIF patients. Furthermore, AHSCT as initial salvage was the strongest independent predictor of survival in our multivariable analysis. This is consistent with a previous report supporting the notion that AHSCT immediately after induction failure was associated with a favorable prognosis in a large, heterogeneous group of relapsed AML patients.[10]

Other groups have suggested a benefit to cytoreduction prior to AHSCT. This entails administration of further salvage chemotherapy with intent to achieve remission or decrease leukemic burden prior to AHSCT. Indeed, fifteen of our patients underwent salvage chemotherapies followed by AHSCT at later date. Unfortunately, their outcomes remained dismal with remission rates and overall survival rates inferior to patients who underwent up-front AHSCT. Similarly, a large number of patients referred for AHSCT may not eventually receive AHSCT due to a variety of reasons as published by Estey et al. [19] Delays or occurrence of infections may further reduce the ability of patients to proceed to AHSCT. Thus, the best chance to receive AHSCT and benefit from it seems to be up-front at failure to primary induction regimen.

These results emphasize the need to explore an intensified second induction followed by AHSCT as initial salvage for patients with PIF AML.

Rationale for Decitabine/CIA/Venetoclax regimen prior to AHSCT

Combination chemotherapy for AML: Emerging role of purine analogs

Anthracyclines have been traditionally combined with cytarabine in AML. Two recent publications highlighted higher response rates and better disease-free survival in both younger and older patients with frontline AML when high-dose daunorubicin (90 mg/m^2/dose) was used in a “7+3” induction combination highlighting the activity of this class of agents for AML therapy.[20, 21] Pautas et al randomized 468 AML patients ages 50 to 70 years to 3 induction anthracycline regimens: idarubicin 12 mg/m^2 daily for 3 days, idarubicin 12 mg/m^2 daily for 4 days, and high-dose daunorubicin 80 mg/m^2 daily for 3 days.[22] The CR rate with idarubicin daily for 3 days was higher than with high-dose daunorubicin (83% vs. 70%). A recent report from the Polish Acute Leukemia Group provides evidence for adding purine analog cladribine to the standard daunorubicin plus cytarabine regimen.[23] In a phase III study, 652 untreated patients (ages 18 to 60 years) with AML were randomized to receive either daunorubicin plus cytarabine (DA) alone,

DA plus fludarabine, or DA plus cladribine. The CR rate was significantly higher in the cladribine arm compared to the DA arm (67.5% vs. 56%; p=0.01). The OS was also better in the cladribine arm compared to DA arm (3-year OS: 45% vs. 33%, p=0.02). The survival advantage of the cladribine arm over the DA arm was observed among patients age 50 years or older (p=0.005), those with initial leukocyte count above 50 x 109/L (p=0.03), and those with unfavorable karyotype (p=0.03).

Clofarabine in AML

Clofarabine (2-chloro-20-fluoro-deoxy-9-b-D-arabinofuranosyladenosine) is a second-generation nucleoside analog ,[31] which was developed as a hybrid molecule to combine the most favorable pharmacokinetic properties of both fludarabine and cladribine and has better stability with higher affinity to deoxycytidine kinase, the rate-limiting step in phosphorylation of nucleosides.[24] Clofarabine acts as an inhibitor of both DNA synthesis and the enzyme ribonucleotide reductase (RNR). Its structural characteristics render it resistant to deamination by adenosine deaminase and phosphorolytic cleavage by bacterial purine nucleoside phosphorylase.[25-28] Single agent clofarabine has demonstrated activity in phase I-II studies in AML.[29,30] As a potent inhibitor of ribonucleotide reductase (RNR) and by means of biochemical modulation, clofarabine is more ideally suited to be incorporated into combinations such as have been tested and validated with cytarabine in AML.[31] We have previously conducted a study combining cytarabine with clofarabine in patients with relapsed/refractory AML.[32] At doses of 1 g/m daily x 5 days for cytarabine and 40 mg/m daily x 5 days for clofarabine, we reported a response rate of 40% (28% complete remission) in 29 patients with a median age of 59 years (18 to 84 years).

Responses extended to patients with primary refractory disease and those with abnormal cytogenetics. Other groups have since followed this lead and published comparable results.[33] We have also reported adaptively randomized study of lower-dose clofarabine (30 mg^2/m IVdaily for 5 days) with or without low-dose cytarabine (20 mg/m SQ daily for 14 days) in previously untreated older patients (older than 60 years) with AML. CR rate was significantly higher with the combination therapy (63% vs. 31%; p=0.025). The combination arm also had improved event free survival (7.1 months vs. 1.7 months; p=0.04), but not overall survival (11.4 months vs. 5.8 months; p=0.1). No excess toxicity was observed in the combination arm.

We have previously explored the combination of clofarabine with cytarabine and anthracycline (CIA regimen). In a phase I study for patients with relapsed/refractory AML, the following doses were established for further evaluation: clofarabine 22.5 mg/m^2 daily x 5 days, idarubicin 6 mg/m^2 daily x 3 days, and cytarabine 0.75 mg/m^2 daily x 5 days.[34] In a recent update of a phase II study of 63 patients treated with CIA regimen,[35] the overall response rate was 38% including 21% CR. Median overall survival was 34 weeks for all patients and 66 weeks for those patients who achieved CR/CRp. Twenty-four patients (38%) were able to proceed with a stem cell transplant. Induction mortality was at 8% and toxicities were manageable.

The role of hypomethylating agents in AML

In the last decade there has been an increasing recognition of the important pathogenetic role of epigenetic changes in leukemogenesis.[36] Aberrant DNA methylation of tumor suppressor genes, leading to their inactivation is an important step in carcinogenesis.[37] Thus, drugs such as DNA methyltransferase inhibitors which lead to DNA hypomethylation may lead to re-expression of tumor suppressor genes. Two such agents, 5-azacytidine and decitabine are currently approved by the United States Food and Drug Administration (FDA) for the treatment of MDS and have demonstrated single-agent activity in AML patients.[38-41] Kantarjian et al. performed a multicenter, randomized, open-label, phase III trial comparing the efficacy and

safety of decitabine with treatment choice (TC, supportive care or cytarabine 20 mg/m^2 per day subcutaneously for 10 consecutive days every 4 weeks) in older patients (age 65 years and above) with newly diagnosed AML and poor- or intermediate-risk cytogenetics.[38] Four hundred eighty five patients were randomly assigned 1:1 to receive decitabine 20 mg/m^2 per day as a 1-hour intravenous infusion for five consecutive days every 4 weeks or TC. The primary analysis showed a nonsignificant increase in median OS with decitabine (7.7 months; 95% CI, 6.2 to 9.2) versus TC (5.0 months; 95% CI, 4.3 to 6.3; p=0.10). Based on this trial results, on September 28, 2012, the European Commission approved decitabine for the treatment of adult patients (age 65 years and above) with newly diagnosed AML, who are not candidates for standard induction chemotherapy.

Combination of hypomethylating agents and chemotherapy in AML

There is preclinical evidence that DNA hypomethylating agents can sensitize tumor cells to cytotoxic chemotherapy.[42-46] Studies in the late 1970's showed that combination of 5-azacytidine and cytarabine was synergistic both in vitro and in vivo, when used sequentially[53] whereas concurrent administration was antagonistic.[47] There studies were, however, done using higher doses of 5-azacytidine (600-1500 mg/m per course), which led to significant non-hematologic toxicities in these earlier clinical trials. It is now known that the effects of 5-azacytidine and decitabine on DNA hypomethylation occur at a significantly lower dose range; and the standard dose of decitabine is 20 mg/m^2 daily for 5 days.[49-52] Qin et al. reported the synergism of decitabine at clinically relevant doses with cytarabine in the HL-60 AML cell line.[46] The authors showed that hypomethylated cells following treatment with decitabine were more likely to undergo apoptosis when treated with cytarabine. Scandura et al. recently reported outcomes of 30 untreated younger (age 60 years and below) patients with intermediate/poor risk AML who received decitabine as a 'priming' agent prior to the conventional '7+3' chemotherapy.[53] Overall a CR rate of 83% was noted (57% with first induction). No excess toxicity was noted with the decitabine priming approach and the toxicity profile was similar to the standard induction. Though there was a prior concern for potential prolonged myelosuppression with this approach, the authors, on the contrary found faster platelet recovery (median 22 days) with the decitabine 'priming' compared to their prior experience with cytarabine-containing induction regimens.[53] The German group has reported preliminary results of a dose-finding study in which 5-azacytidine was used at 2 different dosing regimen (75 mg/m^2 and 37.5 mg/m^2) immediately prior to standard induction with '7+3'.[54] In their preliminary analysis, the tolerability of the 2 arms was similar and they have selected 75 mg/m dose (the standard dosing for 5-azacytidine) as the phase II dose.

Venetoclax has been shown to have significant activity in patients with AML and in combination with other agents may lower remission rate in these patients.

Based on these observations, we hypothesize that the addition of decitabine and venetoclax to our current standard chemotherapy regimen (clofarabine, idarubicin, cytarabine), the DAC-CIA regimen, would be synergistic and would ultimately lead to an improvement in CR rates for AML patients who failed standard induction treatment. We intend to use this more intense regimen to increase the CR rates prior to AHSCT.

Allogeneic hematopoietic stem cell transplantation as up-front treatment modality for PIF AML

Several retrospective studies[55-62] and one prospective report[63] evaluated the role of allogeneic stem cell transplantation for patients with primary refractory AML. The largest single institutional study comes from City of Hope. Fung et al. reported long follow-up results of 68 patients with PIF AML treated with AHSCT. Forty percent of these patients did not receive more than 1 cycle of induction chemotherapy and 40% did not receive a HDAC containing regimen. The median age was 37 years, 82% had a matched related donor transplant and 76% a TBI-based conditioning regimen. The 3-year probability of disease-free survival (DFS) and relapse rate were 31% and 51%. In multivariate analysis (MVA) the only factors associated with worse survival were poor-risk cytogenetics and the use of an unrelated donor for transplantation. The 3-year probability of DFS and relapse were 44% and 37% for patients with intermediate-risk and 18% and 57% for patients with poor-risk cytogenetics.[60]

The Center for International Blood and Marrow Transplantation Research (CIBMTR) published a study on 142 patients with acute leukemia who failed to achieve remission after at least 2 courses of induction chemotherapy who received a matched related allogeneic stem cell transplant. Eighty-eight of these patients had AML. The median bone marrow blast count was 25%. The 3-year probability of DFS was 21%. In this analysis, factors associated with better survival were a bone marrow blasts count <25% (32% vs. 12% survival at 3 years) and less than 3 courses of chemotherapy versus >4.[56]

European Group for Blood and Marrow Transplantation (EBMT) analyzed outcomes of 346 patients with primary refractory AML who underwent matched sibling donor transplantation after a median of 136 days from the initial diagnosis. After a median follow-up of 22 months, the TRM, relapse incidence and leukemia-free survival (LFS) at 2 years were 25%, 57%, and 18%, respectively.[59]

While all these studies reported outcomes of matched sibling donor transplants, Craddock et al. reported recently on a cohort of 168 PIF AML patients from the EBMT database treated with an unrelated donor transplant .[63] The median time to transplant was 5 months, 69% had intermediate-risk cytogenetics, the median BM blasts at transplant was 38.5%, 66% had peripheral blood stem cells, and 78% had myeloablative conditioning. 9% of patients had primary graft failure, grade 3-4 acute GVHD (aGVHD) was 25% and 77% achieved remission post transplant. The leukemia-free survival was 26% at 2 years and 22% at 5 years, while for patients who achieve CR post-transplant was 35% and 31%, respectively. In MVA >2 courses of induction chemotherapy, > 38.5% bone marrow blasts at transplant and patient's negative CMV serostatus was associated with worse outcomes. Based on these 3 factors, a prognostic scoring system was generated, with 5 year OS ranging from 44% when all these factors were absent to 0% when all these risk factors were present. Interestingly, in this study, karyotype did not appear to influence outcomes and, while reduced-intensity chemotherapy (RIC) was associated with better survival than myeloablative (MA) conditioning (5 year DFS 33% vs.17%) this did not reach statistical significant in univariate analysis.[63]

Schmid and colleagues treated prospectively in a multicenter study 103 patients with refractory AML (37 had PIF) with sequential chemotherapy, RIC allogeneic transplantation followed by prophylactic DLI (for patients who did not develop graft-versus-host disease [GVHD]). [62] Sixty percent had an HLA identical donor transplant, 90% a peripheral blood graft, 45.6% poor-risk cytogenetics. The median bone marrow (BM) blasts before salvage chemotherapy was 30%. All patients engrafted the donor cells. After a median follow-up of 25 months, the 4-year LFS was

[62]

30%. In MVA more than 2 courses of prior chemotherapy was the strongest predictor of poor outcomes. TRM was 20%, grade 3-4 aGVHD occurred in 14 patients and 26 patients developed pneumonia, most commonly fungal in 12 patients. TRM was higher with a female donor and after unrelated donor transplantation. Interestingly, DFS was >80% in patients who received prophylactic DLIs (N=17).

Our group[65] reported recently the MDACC experience for patients with PIF AML who failed HDAC induction chemotherapy. Two hundred eighty five patients had PIF and 28 patients receive AHSCT. The median time from induction to transplant was 76 days. The transplanted patients had a median age of 56 years, 19% bone marrow blasts, 21% poor-risk cytogenetics and 64% of the patients had a matched sibling donor for transplant. Eighty two percent achieved complete remission after transplant (vs. 11% with salvage chemotherapy alone). The 3-year overall survival (OS) rate was 39% for patients undergoing transplant (vs. 2% for patients who did not). The clinical incidence (CI) of grade 3-4 aGVHD and chronic GVHD (cGVHD) were 11% and 29%, respectively.[65] Predictors of poor outcomes were high risk cytogenetics and higher percentage of bone marrow blasts at transplant. In MVA AHSCT remained the most significant predictor of disease-free survival.

Rationale for the Study
Up to 30-40% of adults with newly diagnosed AML fail to achieve CR after standard induction chemotherapy, and are deemed primary induction failure. The outcomes of patients with AML who are refractory to induction therapy are dismal, with low response rates to salvage chemotherapy and poor long-term survival (median OS 2.9 months). For this reason, we have provided stopping rules in this protocol allowing high levels of toxic death as long as the success rate is high enough to improve over these historical data. Several studies reported improved survival with up-front AHSCT for PIF AML. Initial salvage with AHSCT had significantly superior outcomes including a response rate of 80-90% and long-term survival of 20-40% of patients.

In this study, we aim to explore the use of intensified induction chemotherapy regimen with decitabine, clofarabine, idarubicin, cytarabine (DCIA) for patients with PIF AML, followed immediately by AHSCT. DCIA should help reduce leukemic burden and improve the success rate of AHSCT for appropriately selected patients.

3.0 Background Drug Information

3.1 Idarubicin
Mechanism of action: Similar to doxorubicin and daunorubicin; inhibition of DNA and RNA synthesis by intercalation between DNA base pairs.

Adverse effects: Cardiovascular: transient EKG abnormalities (supraventricular tachycardia, S-T wave changes, atrial or ventricular extrasystoles); generally asymptomatic and self-limiting; congestive heart failure, dose-related (the relative cardiotoxicity of idarubicin compared to doxorubicin is unclear. Some investigators report no increase in cardiac toxicity at cumulative oral idarubicin doses up to 540 mg/m2; other reports suggest a maximum cumulative intravenous dose of 150 mg/m2); Central nervous system: headache; Dermatologic: alopecia (25% to 30%), radiation recall, skin rash (11%), urticarial; Gastrointestinal: nausea, vomiting (30% to 60%); diarrhea (9% to 22%); stomatitis (11%); GI hemorrhage (30%); Genitourinary:discoloration of urine (darker yellow); Hematologic: myelosupression, primarily leukopenia; thrombocytopenia and anemia. Hepatic: bilirubin and transaminase elevation (44%)

Administration: For I.V. administration only. Do not administer I.M. or SubQ; administer as slow push over 3-5 minutes, preferably into the side of a freely-running saline or dextrose infusion **or** as intermittent infusion over 10-30 minutes into a free-flowing I.V. solution of NS or D5W.

Pharmaceutical Data: Solution, Intravenous, as hydrochloride [preservative free. Store intact vials of solution under refrigeration at 2°C to 8°C (36°F to 46°F). Protect from light. Solutions diluted in D5W or NS for infusion are stable for 4 weeks at room temperature, protected from light. Syringe and IVPB solutions are stable for 72 hours at room temperature and 7 days under refrigeration.

3.2 Cytarabine

Mechanism of action: Cytarabine is an antimetabolite. Cytarabine is cell cycle–specific for the S phase of cell division. Activity occurs as the result of activation to cytarabine triphosphate in the tissues and includes inhibition of DNA polymerase and incorporation of cytarabine into DNA and RNA.

Adverse effects: COMMON - Cardiovascular: thrombophlebitis; Dermatologic: rash, conjunctivitis; Endocrine metabolic: hyperuricemia; Gastrointestinal: anal inflammation, diarrhea, loss of appetite, nausea, stomatitis; ulcer of anus, ulcer of mouth, vomiting; Hematologic: decreased reticulocyte count, megaloblastic anemia; Hepatic: decreased liver function; other: fever. SERIOUS: Central nervous system: cerebellar toxicity (high dose cytarabine); Gastrointestinal: pancreatitis, pneumatosis cyoides intestinalis (high dose cytarabine);Hematologic: anemia, bleeding, leukopenia, thrombocytopenia; Immunologic: anaphylaxis; Neurologic: neuropathy; Renal: kidney disease; Respiratory: pulmonary edema, respiratory distress (high dose cytarabine). Other: sepsis.

Administration: Infuse high-dose therapy over 1 to 3 hours.

Pharmaceutical Data: Solution, injection: 20 mg/mL (25mL); 100 mg/mL (20 mL)

Solution reconstituted, injection: 100 mg, 500 mg or 1000mg

Store intact vials of powder for injection at room temperature of 20°C to 25°C (68°F to 77°F); store intact vials of solution at room temperature of 15°C to 30°C (59°F to 86°F). Solutions for I.V. infusion diluted in D5W or NS are stable for 8 days at room temperature, although the manufacturer recommends administration as soon as possible after preparation. Powder for reconstitution: Reconstitute with bacteriostatic water for injection (for standard-dose).

For IV infusion: Further dilute in 250 to 1000 mL 0.9% NaCl or D5W.

3.3 Clofarabine

Mechanism of action: Clofarabine is a purine nuceloside analog that is metabolized to clofarabine 5'-triphosphate. Clofarabine 5'-triphosphate decreases cell replication and repair and causes cell death. Clofarabine potently inhibits DNA synthesis by inhibiting both DNA polymerase and ribonucleotide reductase. Clofarabine demonstrated the ability to disrupt mitochondrial integrity that results in the release of pro-apoptotic proteins, cytochrome C and apoptosis-inducing factor.

Adverse effects: Common: Cardiovascular: tachycardia, hypotension, flushing, hypertension, edema; Central nervous system: headache, fever, chills, fatigue; Dermatologic: pruritis, rash, palmar plantar erythrodysesthesia syndrome, erythema; Hepatic : ALT increased, AST increased, bilirubin increased; Renal: increased creatinine, hematuria. Other: Hematologic: myelosuppression, infections; Gastrointestinal: nausea/vomiting, diarrhea, mucositis, stomatitis/ pharyngitis, hyperbilirubinemia, increase of SGPT and/or SGOT, abdominal pain or cramping, peritonitis, pancreatitis, liver failure; Dermatologic: skin rash with blisters (particularly hand-foot syndrome), alopecia, Steven-Johnson's syndrome; Systemic: fatigue, asthenia, anorexia, lethargy, malaise, mental status changes/coma, alopecia; Allergic reactions: fever, muscle aches, edema, dyspnea; Cardiology: congestive heart failure; Nephrology: kidney failure; Autoimmune reactions: antiplatelet antibodies, erythema nodosum.

Administration: Infuse over 1 to 2 hours (Faderl, 2008; Kantarjian, 2003; Kantarjian, 2010). Continuous I.V. fluids are encouraged to decrease adverse events and tumor lysis effects. Hypotension may be a sign of capillary leak syndrome or systemic inflammatory response syndrome (SIRS). Discontinue if the patient becomes hypotensive during administration; may consider therapy reinitiation with a 25% dose reduction after return to baseline. Do not administer any other medications through the same intravenous line.

Pharmaceutical data: Solution, Intravenous [preservative free]: Clolar: 1 mg/mL (20 mL). Store intact vials at room temperature of 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). Solutions diluted for infusion in D5W or NS are stable for 24 hours at room temperature. Clofarabine should be diluted with NS or D5W to a final concentration of 0.15-0.4 mg/mL. Manufacturer recommends the product be filtered through a 0.2 micron filter prior to dilution.

3.4 Decitabine

Mechanism of action: Decitabine is a nucleoside analog. It is metabolized intracellularly by the enzyme deoxycytidine kinase and gets incorporated into DNA. It is believed to exert its antineoplastic effects by causing hypomethylation of DNA and direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. Hypomethylation may restore normal function to genes that are critical for differentiation and inhibition of proliferation.

Adverse effects: Myelosuppression, nausea, vomiting, diarrhea, mucositis, skin rash, fatigue, mental status changes/coma, allergic reactions (including fever, muscle aches, edema, dyspnea), congestive heart failure, liver failure, kidney failure, infections, lethargy, malaise, asthenia, alopecia, peritonitis, anorexia, stomatitis/pharyngitis, hyperbilirubinemia, increase of SGPT and/or SGOT, abdominal pain or cramping.

Also reported on decitabine trials, but with the relationship to decitabine still undetermined: allergic reaction, allergic rhinitis, GVHD, atrial fibrillation, cardiac ischemia/infarction, cardiopulmonary arrest, hypotension, left ventricular systolic dysfunction, right ventricular dysfunction, fever, insomnia, rigors/chills, weight loss, pruritus, rash, anal ulcer, ascites, constipation, abdominal distention, esophagitis, GI obstruction, ileus, taste alteration, CNS hemorrhage, GI hemorrhage, lung hemorrhage, nose hemorrhage, petechiae, urinary hemorrhage, cholecystitis, liver failure, infection without neutropenia, opportunistic infection, perianal abscess, head/neck edema, alkaline phosphatase, creatinine, hypercalcemia, hyponatremia, fracture, agitation, CNS ischemia, confusion, depression, dizziness, extrapyramidal/involuntary movement, motor neuropathy, psychosis, seizure, sensory neuropathy, bone pain, headache, muscle pain, urinary pain, acute respiratory distress syndrome (ARDS), bronchospasm, cough, dyspnea, hiccups, pneumonitis/pulmonary infiltrates, cystitis.

Administration: Infuse over 1-3 hours. Premedication with antiemetics is recommended.

Pharmaceutical data: Injection, powder for reconstitution: Dacogen™: 50 mg

Store vials at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). Solutions diluted for infusion may be stored for up to 7 hours under refrigeration at 2°C to 8°C (36°F to 46°F) if prepared with cold infusion fluids Vials should be reconstituted with 10 mL SWFI to a concentration of 5 mg/mL. Immediately further dilute with 50-250 mL NS, D5W, or lactated Ringer's to a final concentration of 0.1-1 mg/mL. Solutions not administered within 15 minutes of preparation should be prepared with cold (2°C to 8°C [36°F to 46°F]) infusion solutions.

3.5 Busulfan

Mechanism of action: Interferes with DNA replication and transcription of RNA through DNA alkylation, and ultimately results in the disruption of nucleic acid function.

Side effects: Dose limiting toxicity is expected to be hematological when used without stem cell support. Other toxicities seen frequently following high-dose busulfan in preparative regimens for bone marrow transplantation include: Veno-occlusive disease (VOD), nausea, vomiting, pulmonary fibrosis, seizures, rash, and an Addison's-like syndrome.

Human Pharmacology: Limited pharmacology data are available for the parenteral formulation to be used in this study and is detailed in the evaluation of IV Busulfan (Bu) in a Phase II Trial using IV Bu at 0.8 mg/kg BW given over 2 hr every 6 hr for a total of 16 doses (37) and when administered once daily for 4 days at a dose of 130 mg/m2 in combination with Flu (29). The pharmacokinetic data suggests that the plasma decay of the formulation fits an open one-compartment model with linear pharmacokinetics in the dose range of 12 mg-130 mg/m2. Based on studies of oral Bu, the drug is reported to be extensively metabolized; twelve (12) metabolites have been isolated, but most have not been identified. The drug is slowly excreted in the urine, chiefly as methanesulfonic acid. Ten to fifty percent (10-50%) of a dose is excreted as metabolites within twenty-four (24) hours (38).

Administration: Test doses of busulfan should be infused IV over 45 to 60 minutes.

Therapeutic doses of IV busulfan should be infused over 3 hours via central line. Flush line before and after each infusion with 5 mL D5W or NS. Do not use polycarbonate syringes or filters for preparation or administration

Pharmaceutical data: Injection: 6 mg/mL (10 mL). Store intact vials under refrigeration at 2°C to 8°C (36°F to 46°F). Solutions diluted in sodium chloride (NS) injection or dextrose 5% in water (D5W) for infusion are stable for up to 8 hours at room temperature (25°C [77°F]); the infusion must also be completed within that 8-hour timeframe.

3.6 Fludarabine

Mechanism of Action: After phosphorylation to fluoro-ara-ATP the drug appears to incorporate into DNA and inhibit DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis.

Pharmaceutical Data: Each vial contains 50 mg lyophilized drug, to be reconstituted with 2 ml sterile water to a solution that is 25 mg/ml for IV administration.

Known Side Effects and Toxicities: pancytopenia, immunosuppression, autoimmune hemolytic anemia has (rarely) been reported, and recurred when patients were retreated with the drug. Nausea, vomiting, anorexia, weakness. From the CNS:
agitation, visual disturbances, confusion, coma, peripheral neuropathies have been reported. With high dose use confusion, blindness, coma and death have been reported.
It may cause red blood cells to burst.

Special Precautions: As for other antineoplastic agents Fludarabine should be handled by trained personnel using procedures for proper handling. The use of gloves and protective glasses is recommended to avoid exposure upon accidental spillage.

Human Safety and Pharmacology: The half-life of the activated compound is approximately 10 hours, but the pharmacology is incompletely understood. Excretion is impaired in patients with impaired renal function.

3.7 Cyclophosphamide

Mechanism of Action: Cyclophosphamide prevents cell division by cross-linking DNA strands and decreasing DNA synthesis. It is cell cycle phase non-specific. Cyclophosphamide also possesses potent immunosuppressive properties. It is a pro-drug metabolized by the liver to active metabolites.

Known Side Effects: Hematologic: Leukopenia, anemia, alopecia. GI: Nausea, vomiting, increased AST, ALT, mucositis, diarrhea. Neurologic: Headache, dizziness. Cardiovascular: Cardiomyopathy, non-specific ST changes on EKG. At doses greater than 200 mg/kg, Cy can cause fatal myocardial necrosis with clinical heart failure. Renal: Hemorrhagic cystitis, SIADH, fluid retention. Cy has anti-diuretic effect usually counteracted by furosemide administration. GU: Hemorrhagic cystitis. Hematuria is not uncommon at this dose level, but is usually not symptomatic or severe unless there is inadequate diuresis. An occasional patient will get severe cystitis despite prophylactic measures. Other: teratogenic, may cause secondary neoplasms, anaphylaxis (rare).

Special Precautions: As for other antineoplastic agents, Cyclophosphamide should be handled by trained personnel using procedures for proper handling. The use of gloves and protective glasses is recommended to avoid exposure upon accidental spillage.

Pharmaceutical data: Injection powder for reconstitution: 500 mg, 1 g, 2

Store intact vials of powder at ·25·C (77·F). Exposure to excessive temperatures may cause active ingredient to melt (vials with melting may have a clear to yellow viscous liquid which may appear as droplets); do not use vials with signs of melting. Reconstituted solutions in normal saline (NS) are stable for 24 hours at room temperature and for 6 days refrigerated at 2·C to 8·C (36·F to 46·F). Solutions diluted for infusion in 1/2NS are stable for 24 hours at room temperature and for 6 days refrigerated; solutions diluted in D5W or D5NS are stable for 24 hours at room temperature and for 36 hours refrigerated.

Injection powder for reconstitution: Reconstitute with 25 mL for a 500 mg vial, 50 mL for a 1000 mg vial, or 100 mL for a 2000 mg vial to a concentration of 20 mg/mL using NS only for direct I.V. push, or NS or SWFI for I.V. infusion; swirl gently to mix. For I.V. infusion, further dilute for infusion in D5W, 1/2NS, or D5NS, to a minimum concentration of 2 mg/mL.

3.8 Mesna

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazophosphorines (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no

antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazophosphorines. At the doses used for uroprotection, mesna is virtually non-toxic. However, adverse effects which may be attributable to mesna include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue.

3.9 Antithymocyte globulin (ATG) (Thymoglobulin® , Rabbit antithymocyte globulin, Genzyme Corporation) will be used as an in vivo immunosuppression.

Mechanism of action: Possible mechanisms by which Thymoglobulin may induce immunosuppression *in vivo* include: T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Thymoglobulin is thought to induce T-cell depletion and modulation by a variety of methods, including Fc receptor-mediated complement-dependent lysis, opsonization and phagocytosis by macrophages, and immunomodulation leading to long term depletion via antibody dependent cell-mediated cytotoxicity and activation induced cell death, commonly referred to as apoptosis.

Administration: The recommended route of administration is intravenous infusion through an in-line 0.22 micron filter into a high-flow vein. Thymoglobulin should be infused over a minimum of 6 hours for the first infusion and over at least 4 hours on subsequent days of therapy.

Known side effects and toxicities: The most common adverse reactions are fever, chills, leukopenia, thrombocytopenia, rashes, systemic infections, abnormal renal function tests, and serum sickness-like symptoms. Other reported side effects are arthralgia, chest and/or back pain, diarrhea, dyspnea/apnea, nausea and vomiting.

At the time of study entry, all patients will have HLA typing done, including first degree relatives.

3.10 Venetoclax

Venetoclax (VEN) is a potent and selective small-molecule inhibitor of Bcl-2 that has demonstrated cell-killing activity against a variety of leukemia cell lines, primary patient samples and leukemia stem/progenitor cells.

Mechanism of action: Venetoclax binds with high affinity to Bcl-2 (>1,000-fold higher than Bcl-XL). Venetoclax has demonstrated potent killing of AML cell lines and leukemic stem/progenitor cells ex vivo.

Pharmacokinetics and metabolism:
The pharmacokinetics of venetoclax was evaluated in mice, rats, monkeys, and dogs. Venetoclax pharmacokinetic (PK) profile was characterized by low plasma clearance and low to moderate volumes of distribution. Half-lives ranged from 2.2 hours in monkeys to 12 hours in dogs. Venetoclax demonstrated high protein binding to human, rat, dog, and monkey plasma proteins (99.9%). Blood to plasma ratios showed that venetoclax does not partition preferentially into the red blood cells. Following oral administration of venetoclax to nonclinical species and humans, parent compound and metabolites were mainly cleared via biliary excretion and fecal elimination, with minimal renal clearance.

Venetoclax is predominantly metabolized by cytochrome P450 (CYP) 3A4 (CYP3A4) in vitro.

In vitro studies indicated that venetoclax is not an inhibitor or inducer of CYP1A2, CYP2B6, CYP2C19, CYP2D6, or CYP3A4 at clinically relevant concentrations. Venetoclax is a weak inhibitor of CYP2C8, CYP2C9, and UGT1A1 in vitro, but it is not predicted to cause clinically relevant inhibition due to high plasma protein binding.

Administration: Venetoclax is administered orally at 400 mg PO once a day for 14 days (days 1-14) OR 200 mg PO once a day on days 1-4 for patients on concomitant second-generation azole due to drug-drug interaction.

Known side effects: The most common adverse drug reactions across all indications are nausea, diarrhea, hematological effects, and serious and/or opportunistic infections. Hematologic effects include neutropenia/febrile neutropenia, thrombocytopenia, anemia, and lymphopenia. Upper respiratory tract infections are among the most common infections. TLS is an important identified risk and is predominantly seen in the CLL population with high tumor burden. Based on pre-clinical data, decreased spermatogenesis has been identified as a potential risk for venetoclax. Most of these side effects are of limited concern in patients undergoing allogeneic transplantation.

4.0 Patient Eligibility

4.1 Inclusion criteria

1. Patients age 18-60 years.
2. Patients with diagnosis of AML, judged primary refractory after up to 2 courses of AML induction therapy (> 5% blasts on day 21 (+/-7 days) bone marrow aspirate and/or biopsy from the beginning of induction chemotherapy, up to 42 days).
3. Eastern Cooperative Oncology Group (ECOG) Performance Status </= 2.
4. Adequate major organ function:, defined as:
   a) Serum creatinine </= 3 mg/dL;
   b) Total bilirubin </= 2.5 mg/dL;
   c) ALT (SGPT) </= 3 x ULN or </= 5 x ULN if related to disease;
   d) Cardiac ejection fraction >/= 40% (by either ECHO or MUGA).
5. Willingness to have an allogeneic transplant.
6. Patient or patient's legal representative able to provide written informed consent.

4.2 Exclusion Criteria

1. HIV positive; active hepatitis B or C.
2. Uncontrolled active infections (viral, bacterial, and fungal); the Study Chair will be the final arbiter of this criterion.
3. Patients with active secondary malignancy unless approved by the Study Chair.
4. Liver cirrhosis.
5. Active CNS involvement within the previous 2 months.
6. Prior induction therapy with DAC + CIA.
7. Positive pregnancy test in a woman with child bearing potential defined as not post-menopausal for 12 months or no previous surgical sterilization.
8. Breast feeding women.
9. Men must agree not to father a child and agree to use a condom if his partner is of child bearing potential.
10. Inability to comply with medical therapy or follow-up

5.0 Criteria to Proceed to Transplant

Patients are required to meet the following criteria to proceed to AHSCT:

5.1 **Donor criteria**: Availability of a donor either an HLA matched sibling donor (MSD) or a haploidentical (5-9/10 HLA matched); alternatively a 8/8 HLA matched unrelated donor (MUD) by high resolution typing is immediately available;

5.2 **Disease criteria** (see schema, Section 9.3): Day 21 (+/-7 days) bone marrow aspiration or biopsy from the beginning of salvage DCIA:

a. In complete **morphologic remission** with <5% bone marrow blasts, or
b. **Aplastic** (<10% bone marrow cellularity), and cytopenic with an absolute neutrophil count (ANC) less than 1,000/μL, or
c. **Low disease burden** with <30% BM blasts, with recovery of peripheral blood (PB) WBC (ANC>1,000/μL) and <5% circulating blasts.

5.3 **Adequate organ function criteria**:

a. Serum creatinine clearance >/= 50 ml/min (calculated by Cockroft-Gault formula, using actual weight);
b. Total bilirubin </= 2 times upper limit of normal (x ULN) (3 x ULN if considered to be due to leukemic involvement or Gilbert's syndrome);
c. Alanine aminotransferase (ALT) </= 3 x ULN (5.0 x ULN if considered to be due to leukemic involvement);
d. LVEF >/= 40% on ECHO or MUGA;
e. DLCO >/= 50% predicted after correction for hemoglobin (must be performed in patients with history of smoking or lung disease;); DLCO may be omitted in patients without history of pulmonary disease if approved by the Study Chair.

5.4 **No active infection**:

Patients should be afebrile. If present, pulmonary infiltrates or other sites of infection must be improving on antibiotics. Patients should not require oxygen. Study Chair will be the arbiter of this criterion.

6.0 Recommended Donor Selection

Preference for donors will be as follows:

1. Matched sibling donor (MSD);
2. A haploidentical donor </= 70 years old (at least 5/10 HLA matched first degree relative);
3. Matched unrelated donor (MUD) defined as 8/8 human leukocyte antigen (HLA) matched at HLA-A,-B,-C,-DRB1. The identified MUD should be already HLA typed by high resolution in the Registry and ready available to donate stem cells within a month after identification.

7.0 Pretreatment Evaluation - Pre Salvage

7.1 Medical history, physical examination and concomitant medication within 14 days of treatment start.

7.2 CBC, differential, platelet count and chemistry panel (at least including BUN, creatinine, SGPT and/or SGOT, total bilirubin, LDH, electrolytes, uric acid) within 7 days of treatment start.

7.3 Bone marrow aspirate and/or biopsy with appropriate flow cytometry within 4 weeks of

treatment start (and cytogenetic evaluation within 3 months of treatment start). For patients with evidence of leukemia in the peripheral blood, the bone marrow may be omitted after discussion and approval with the principal investigator.
7.4 Echocardiography or MUGA scan to assess cardiac ejection fraction within 8 weeks of treatment start.
7.5 For women of childbearing age, a negative serum and/or urine pregnancy test is required within 7 days of treatment start.

8.0 Pretreatment Evaluation - Pre-Transplant

Evaluation Prior to Transplant (baseline):
Standard work up for transplant as well as disease assessment is done prior to study entry as part of diagnostic or routine pre-transplant evaluation. A bone marrow biopsy is required within 2 weeks of proceeding to transplant to assess disease status.

9.0 Treatment Plan

9.1 General plan/schema:

Prior to initiating chemotherapy in this study, all toxicities from prior systemic chemotherapy must be resolved at least to grade 1. Flt3 or TKI inhibitors as well as intra-thecal therapy, nonmyelosuppressive agents, low dose cytarabine, hydroxyurea are permitted if indicated to control active leukemia, but must be stopped at least 5 days prior to administering the PK-test dose of IV Busulfan to avoid pharmacologic interference with IV Busulfan.

Enrolled patients will undergo salvage treatment first with the DCIA regimen. Those meeting the criteria to proceed will receive the conditioning regimen followed by AHSCT. Patients not meeting the criteria for AHSCT will continue best available therapy at the discretion of their attending physician.

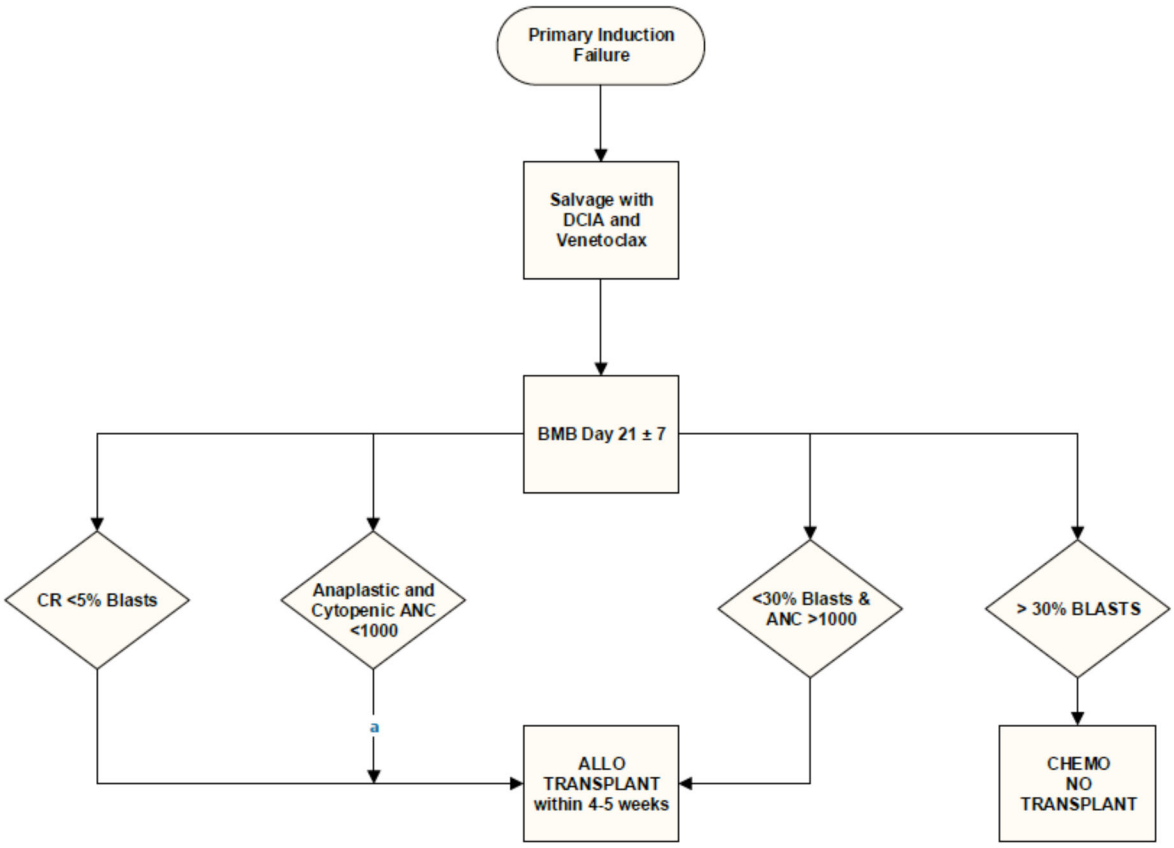
9.2 DCIA/Venetoclax salvage chemotherapy (Appendix A):

Decitabine 20 mg/m2 IV over 1 hour daily for 5 days (days 1-5)
Clofarabine 15 mg/m2 IV over approximately 1 hour daily for 4 days (days 6-9)
Idarubicin 10 mg/m2 IV over approximately 30 minutes daily for 3 days (days 6-8)
Cytarabine 1 g/m2 IV over approximately 2 hours daily for 5 days (days 6-10)
Venetoclax 400 mg PO once a day for 14 days (days 1-14) OR 200 mg PO once a day on days 1-4 for patients on concomitant second-generation azole due to drug-drug interaction.

Idarubicin will follow clofarabine by approximately 1 to 2 hours and cytarabine will follow clofarabine by approximately 3 to 6 hours. Venetoclax should be taken once a day with a meal or water. All chemotherapeutic agents will be dosed, prepared, and administered according to MDACC institutional guidelines. BSA will be recalculated (using actual weight) prior to each subsequent course. A bone marrow will be performed on day 21 (+/- 7 days) of DCIA.

The treating Leukemia Department physician will determine when the second course of DCIA salvage chemotherapy will begin.

9.3 AHSCT:
Will be performed as soon as possible after day 21 (+/- 7 days) BM biopsy post salvage treatment with the DCIA. The goal is to take the patients to transplant within 4-5 weeks after the beginning of salvage treatment with DCIA. The maximum time to proceed to transplant is 8 weeks from the beginning of DCIA.



9.4 Conditioning Regimens and AHSCT:

Patients eligible for transplantation will receive conditioning with intravenous (IV) busulfan plus fludarabine (Flu), clofarabine (Clo), +/- 2 Gy of total body irradiation (TBI). All MUD recipients will receive ATG as part of the conditioning regimen. Graft versus host disease (GVHD) prophylaxis for matched related and unrelated donor transplants will consist of tacrolimus and methotrexate, while haploidentical transplants will receive tacrolimus and mycophenolate mofetil as per departmental guidelines.

Acetaminophen should not be used between D-10 (starting 24 hours before the test dose of IV Busulfan) and Day 0, since there is a major interference between these drugs and the metabolism of Busulfan (Bu) which is likely to contribute in a major way to cause serious liver damage.

Other drugs known to interfere with the metabolism of Flu and/or Bu should not to be concomitantly used during the chemotherapy administration up to and including the day of transplantation if possible. In particular, this pertains to drugs that are known effective inhibitors of, or inducers of the hepatic cytochrome P450-system, such as primidone, voriconazole, itraconazole (and related antifungal drugs), and metronidazole as well as tyrosine-kinase inhibitors. Such agents should be omitted for at least 5 days prior to the test dose of IV Bu or to admission, whichever comes first, for transplantation on this program since these agents have well described interference with busulfan metabolism. They can be resumed starting one day following the stem cell transplant procedure. Patients with prior fungal infections who require azole treatment may continue these agents at a constant dose during the Busulfan test dose and subsequent preparative regimen.

Busulfan test dose:
Busulfan test dose can be administrated in the outpatient setting prior to admission for the first therapeutic Busulfan dose or given in the inpatient setting on Day -8. Pharmacokinetic-guided (PK-guided) treatment: The Bu “test dose” of 32 mg/m^2 will be based on **actual bodyweight**.

This Bu dose will be given IV over 45 minutes (for patients up to age 50) and over 60 minutes (for patients age 51 to 60 or PS 2 or greater) by controlled-rate infusion pump.

Busulfan adjusted dose determined to achieve a systemic exposure represented by an average daily AUC of 5000 µMol-min ± 5% for the entire 4-day treatment period is administered to all patients up to age 50 who have ECOG performance status 0 or 1. The target AUC is 4,000 µMol-min ± 5% for patients ages 51-60 or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose (for the target AUC 5000) and 100 mg/m2 (for target AUC 4000).

Fludarabine/Clofarabine/Busulfan:

The nucleoside analogs Fludarabine and Clofarabine will be dosed per actual body weight/actual body surface area. No arbitrary dose adjustment(s) based on a perceived need for using adjusted body weight/body surface area will be allowed for fludarabine and clofarabine, since insufficient data regarding the impact of modification(s) on engraftment and toxicity/disease-control are available for the use of nucleoside analogs in high-dose conditioning therapy. Flu and Clo must be administered IV once daily by a controlled-rate pump.

Fludarabine administration:

Fludarabine is administered over 1 hour via controlled rate pump at a dose of 10 mg/m^2 in 100 ml of NS on each of four (4) consecutive days (days -6 through -3). Intravenous fluids should be administered at a rate of that is ≥ 65 mL/m2/hour starting the evening before the start of Fludarabine, through twenty-four (24) hours after the last dose of Busulfan.

Clofarabine administration:

Clofarabine will be infused over 1 hour by controlled rate pump and be dosed per actual body weight/actual body surface area. Clofarabine is administered at a dose of 40 mg/m^2 diluted in NS to produce a final concentration of 0.4 mg/mL, and infused on each of four (4) consecutive days (days -6 through -3). The doses of Clofarabine are to follow immediately after Fludarabine administration and prior to Busulfan on days -6 to -3 respectively. Intravenous fluids should be administered at a rate of that is ≥ 65 mL/m2/hour starting the evening before the start of this chemotherapy, through twenty-four (24) hours after the last dose of Bu.

Busulfan administration:

The PK-guided daily high-dose Bu dose(s) will be started immediately upon completion of the daily Clofarabine doses. The Busulfan doses will be diluted in normal saline or 5% dextrose in water and administered daily by controlled rate infusion pump. Busulfan is administered at the dose calculated to achieve a systemic exposure dose of 5000 µMol-min in normal saline IV every twenty-four (24) hours for four (4) consecutive days (days -6 to -3), starting immediately after the completion of Clo. The Bu dose on days -6 to -3 will be based on the pharmacokinetic studies to target an AUC of 4,000 µMol-min ± 5% for patients 51-60 years of age or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m dose. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose.

The PK adjusted dose of Bu=Target AUC x Bu mol. wt (0.2463) x Bu gross the measured clearance normalized to body surface area (L/min) + the dose remaining in the IV line (priming dose).

D-3 to D-1 Anti-thymocyte globulin administration:

Patients in both groups who receive a graft from an unrelated donor will receive Thymoglobulin; 2 mg/kg on day –3 and day –2. On day -3, this will be administered after the chemotherapy is complete. Dose will be based on actual body weight.

Post transplant cyclophosphamide administration with Mesna:

Premedication: Patients will receive a dose of Mesna 10 mg/kg IVPB just prior to the first dose of cyclophosphamide (Cy), that will be repeated every 4 hours for a total of ten (10) doses. Patients will also receive ondansetron (or a comparable anti-emetic) prior to each dose of Cy.

Patients will receive Cy on days + 3 and + 4 at a dose of 50 mg/kg per dose. Patients weighing within 20% above their ideal body weight will be dosed according to actual body weight.

Patients weighing more than 20% above their ideal body weight will be dosed according to the adjusted body weight. Formula to calculate adjusted body weight: Adjusted BW (Kg) = IBW + 0.5 (Actual body weight-IBW). The first dose of cyclophosphamide must be administered 60 to 72 hours following the start of marrow infusion.

Cy will be mixed in D5W to a maximum concentration of 20mg/ml and given IV over 3 hours via pump. Patients should be well hydrated. It is recommended to receive IV Fluids at a rate of 2 ml/kg/hour or max of 150 ml/hour starting the morning (4AM on day+3 and +4) of each dose of Cy (minimum of 4 hours before each dose) and continued for a minimum of 6 hours after each dose.

9.5 Treatment Plans for Matched Sibling Donor Transplants

9.5.1 Treatment Plan for Matched Sibling Donor Transplants - **Inpatient** busulfan test dose (Appendix B.)

-9	Admit / IV Hydration
-8	Test dose Busulfan 32 mg/m2 IV (PK sampling)
-7	Rest / PK Analysis
-6	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)** (PK Sampling)
-5	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-4	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-3	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-2	Rest
-1	Rest
0	**Peripheral blood stem cell infusion**

** Busulfan adjusted dose determined to achieve a systemic exposure represented by an average daily AUC of 5,000 µMol-min ± 5% for the entire 4-day treatment period is administered to all patients up to age 50 who have ECOG performance status 0 or 1. The target AUC is 4,000 µMol-min ± 5% for patients ages 51-60 or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose (for the target AUC 5,000) and 100 mg/m^2 (for target AUC 4,000).
GVHD prophylaxis: Tacrolimus / methotrexate per departmental guidelines.
Filgrastim per SCTCT Department guidelines.

9.5.2 Treatment Plan for Matched Sibling Donor Transplants - **Outpatient** busulfan test dose (Appendix C.)

	Test dose Busulfan 32 mg/m2 IV (PK sampling)
-7	Admit / IV Hydration
-6	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)** (PK Sampling)
-5	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-4	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-3	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-2	Rest
-1	Rest
0	**Peripheral blood stem cell infusion**

** Busulfan adjusted dose determined to achieve a systemic exposure represented by an average daily AUC of 5,000 µMol-min ± 5% for the entire 4-day treatment period is administered to all patients up to age 50 who have ECOG performance status 0 or 1. The target AUC is 4,000 µMol-min ± 5% for patients ages 51-60 or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose (for the target AUC 5,000) and 100 mg/m^2 (for target AUC 4,000).
GVHD prophylaxis: Tacrolimus / methotrexate per departmental guidelines.
Filgrastim per SCTCT Department guidelines.

9.6 Treatment Plans for Haploidentical Transplants

9.6.1 Treatment Plan for Haploidentical Transplants - **Inpatient** busulfan test dose (Appendix D.)

-9	Admit / IV Hydration
-8	Test dose Busulfan 32 mg/m2 IV (PK analysis)
-7	Rest / PK Analysis
-6	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)** (PK Sampling)
-5	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-4	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-3	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-2	2 Gy TBI
-1	Rest
0	**Bone marrow stem cell infusion (peripheral blood as alternative)**
+3	Cyclophosphamide 50 mg/kg IV
+4	Cyclophosphamide 50 mg/kg IV

** Busulfan adjusted dose determined to achieve a systemic exposure represented by an average daily AUC of 5,000 µMol-min ± 5% for the entire 4-day treatment period is administered to all patients up to age 50 who have ECOG performance status 0 or 1. The target AUC is 4,000 µMol-min ± 5% for patients ages 51-60 or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose (for the target AUC 5,000) and 100 mg/m^2 (for target AUC 4,000).
Tacrolimus: Start 0.015 mg/kg/day IV or PO on Day +5 and mycophenolate mofetil 15 mg/kg/dose IV/PO TID from day +5 to D+100 or otherwise indicated.
Filgrastim per SCTCT Department guidelines.

9.6.2 Treatment Plan for Haploidentical Transplants - **Outpatient** busulfan test dose (Appendix E.)

	Test dose Busulfan 32 mg/m2 IV (PK analysis)
-7	Admit / IV Hydration
-6	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)** (PK Sampling)
-5	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-4	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-3	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV (adjusted)**
-2	2 Gy TBI
-1	Rest
0	**Bone marrow stem cell infusion (peripheral blood as alternative)**
+3	Cyclophosphamide 50 mg/kg IV
+4	Cyclophosphamide 50 mg/kg IV

** Busulfan adjusted dose determined to achieve a systemic exposure represented by an average daily AUC of 5,000 µMol-min ± 5% for the entire 4-day treatment period is administered to all patients up to age 50 who have ECOG performance status 0 or 1. The target AUC is 4,000 µMol-min ± 5% for patients ages 51-60 or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose (for the target AUC 5,000) and 100 mg/m^2 (for target AUC 4,000).
Tacrolimus: Start 0.015 mg/kg/day IV or PO on Day +5 and mycophenolate mofetil 15 mg/kg/dose IV/PO TID from day +5 to D+100 or otherwise indicated.
Filgrastim per SCTCT Department guidelines.

9.7 Treatment Plans for MUD Transplants

9.7.1 Treatment Plan for MUD Transplants - **Inpatient** busulfan test dose (Appendix F.)

-9	Admit / IV Hydration
-8	Test dose Busulfan 32 mg/m2 IV (PK sampling)
-7	Rest / PK Analysis
-6	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted) (PK Sampling)
-5	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted)
-4	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted)
-3	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted) / Thymoglobulin 2.0 mg/Kg IV
-2	Thymoglobulin 2.0 mg/Kg IV
-1	Rest
0	**Peripheral blood stem cell infusion**

** Busulfan adjusted dose determined to achieve a systemic exposure represented by an average daily AUC of 5,000 µMol-min ± 5% for the entire 4-day treatment period is administered to all patients up to age 50 who have ECOG performance status 0 or 1. The target AUC is 4,000 µMol-min ± 5% for patients ages 51-60 or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose (for the target AUC 5,000) and 100 mg/m^2 (for target AUC 4,000).
GVHD prophylaxis: Tacrolimus / methotrexate per departmental guidelines.
Filgrastim per SCTCT Department guidelines.

9.7.2 Treatment Plan for MUD Transplants - **Outpatient** busulfan test dose (Appendix G.)

	Test dose Busulfan 32 mg/m2 IV (PK sampling)
-7	Admit / IV Hydration
-6	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted) (PK Sampling)
-5	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted)
-4	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted)
-3	Fludarabine 10 mg/m2 IV / Clofarabine 40 mg/m2 IV / Busulfan IV** (adjusted) / Thymoglobulin 2.0 mg/Kg IV
-2	Thymoglobulin 2.0 mg/Kg IV
-1	Rest
0	**Peripheral blood stem cell infusion**

** Busulfan adjusted dose determined to achieve a systemic exposure represented by an average daily AUC of 5,000 µMol-min ± 5% for the entire 4-day treatment period is administered to all patients up to age 50 who have ECOG performance status 0 or 1. The target AUC is 4,000 µMol-min ± 5% for patients ages 51-60 or younger patients with ECOG performance status 2. If pharmacokinetic analysis cannot be completed for any reason, Bu will be given in a dose of 130 mg/m^2 dose (for the target AUC 5,000) and 100 mg/m^2 (for target AUC 4,000).
GVHD prophylaxis: Tacrolimus / methotrexate per departmental guidelines.
Filgrastim per SCTCT Department guidelines.

9.7.3 Reduced Intensity Treatment Plan (Appendix H.)

-6	Admit / IV Hydration
-5	Melphalan 100 mg/m2 IV / Fludarabine 40 mg/m2 IV
-4	Fludarabine 40 mg/m2 IV
-3	Fludarabine 40 mg/m2 IV
-2	Fludarabine 40 mg/m2 IV
-1	TBI 200 cGY
0	**Peripheral blood stem cell infusion**
+5	Start Tacrolimus at 0.015 mg/kg CIV, changed to PO for 6 months and MMF 15 mg/kg PO (divided in TID dosing) until Day 100
+7	Start G-CSF 5 mcg/kg/day SQ (rounded as per institutional guidelines)

9.8 Stem Cell Infusion

Fresh or cryopreserved bone marrow or peripheral blood progenitor cells will be infused on day 0. The goal is to infuse 4 X 10^6 CD34+ cells/kg if PB or >3.0 X 10^8 marrow mononuclear cells/kg if bone marrow. Premedication for the infusions will be per standard SCTCT department procedures for allogeneic and matched unrelated transplants.

For haploidentical transplant receiving post-transplant cyclophosphamide, corticosteroids are not to be used as a premedication for stem cell infusion.

If the patient cannot receive the peripheral blood stem cell infusion by the 8th week, the patient will continue with the best alternative treatment in the Leukemia Department.

9.9 Supportive Care

Prophylaxis and Supportive Care as per standard practice in patients receiving allogeneic transplant and SCTCT Guidelines.

Seizure prophylaxis during busulfan is per SCTCT departmental standard practice.

10.0 Evaluation During Study - Post Salvage

10.1 Physical exam prior to every cycle.
10.2 CBC, differential, and platelet count twice weekly during induction and reinduction then at least weekly during consolidation. The differential may be omitted when the WBC count is ≤ 500/uL.
10.3 Chemistry panel (at least including electrolytes, BUN, creatinine, SGPT and/or SGOT, total bilirubin) at least weekly during induction and reinduction then every two weeks during consolidation.
10.4 CBC, differential, platelet count, creatinine, and bilirubin prior to each consolidation course.
10.5 Bone marrow aspirate and/or biopsy starting day 33 of induction and reinduction (+/- 7 days) and every 2 weeks (+/- 7 days) thereafter until remission or non-response (Day 33 is listed instead of usual day 28 due to 5 days of decitabine treatment prior to chemotherapy). No bone marrow is necessary if non-response or progressive disease can be diagnosed from peripheral blood evaluation, or, in patients with a WBC ≤ 300/uL, if the bone marrow test is considered non-contributory by the Investigator. Cytogenetic studies need to be repeated only if abnormal prior to study. Further bone marrow tests as indicated by development of peripheral blood counts.
10.6 Marrow aspirates would be repeated every 2-3 cycles during consolidation phase.

11.0 Evaluation During Study - Post-Transplant

For the patient having a transplant, the active treatment period is defined as the the day of admission through BMT Day +30. The follow-up period is defined as BMT Day +31 until 2 years after cell product infused.

11.1 The following are standard evaluations performed to document engraftment and disease status. These evaluations should be performed at approximately 1 month (+/- 1 week), 3 months (+/- 2 weeks), 6 months (+/- 2 weeks), and 12 months (+/-1 month) post-transplant. If indicated these studies may be done at other timepoints to evaluate potential relapse of the underlying malignancy, which can replace the nearest planned assessment.

11.1.1 Bone marrow biopsy and aspirate with cytogenetics.

11.1.2 Chimerism studies from peripheral blood performed on separated T-cells and myeloid cells

11.1.3 In addition to above time-points, peripheral blood T-cell subsets and B cell immune reconstitution will be performed at approximately 2 months (+/- 1 week).

11.1.4 At each visit, a physical examination and adverse event documentation will be completed.

11.1.5 GVHD and adverse events assessment: while the patient is in house will be conducted by the SCTCT in-patient team on a daily basis and thereafter at each outpatient visit.

11.2 The following SOC lab tests are to be performed as frequently as clinically indicated: CBC, differential, platelets, SGPT, calcium, glucose, uric acid, magnesium, serum bilirubin, BUN and creatinine, serum protein, albumin, alkaline phosphatase, electrolytes, urinalysis, tacrolimus levels and CMV antigenemia.

11.3 After the first year, patients will be followed-up for one additional year to assess survival, GVHD and disease status.

12.0 Adverse Events and Reporting Requirements

12.1 The salvage treatment delivered in the Leukemia Department is considered standard of care.
The adverse events will not be captured and recorded for this trial. The investigational part of this trial is adding the allogeneic transplant earlier in the AML treatment.

12.2 Stem Cell Transplatation-specific Adverse Event Recording and Reporting Guidelines:
These guidelines will be followed by the Stem Cell Transplantation and Cellular Therapy Department when the patient begins conditioning regimen for transplantation.

Assessment of the Adverse Events Severity.
The severity of the adverse events (AEs) will be graded according to the Common Terminology Criteria v4.0 (CTCAE).

Events not included in the CTCAE chart will be scored as follows:

General grading:
Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.
Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.
Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.
Grade 4: Life Threatening: discomfort that represents immediate risk of death

Grading for specific syndromes:
Veno-occlusive disease (VOD):
Grade 3: Bili >2mg/dl with at least two of the following: increased weight >4% from baseline, ascites or hepatomegaly
Grade 4: pulmonary and or renal failure

Pulmonary events not caused by CHF (interstitial pneumonitis (IP), pulmonary hemorrhage (DAH):
Grade 1: CXR showing mild infiltrates or interstitial changes
Grade 2: mild SOB
Grade 3: requires supplemental oxygen, or is a documented infection
Grade 4: requires intubation

Transplant related microangiopathy:
Grade 1: No treatment required
Grade 2: Requires steroids and/or plasma transfusions
Grade 3: Requires plasma exchange

Cytokine storm or engraftment syndrome:

Grade 1: No treatment required
Grade 2: Treatment required
Grade 3: Organ dysfunction
Grade 4: Total Bilirubin >5

Hemorrhagic Cystitis:
Grade 1: minimal or microscopic bleeding/pain
Grade 2: gross bleeding/pain and spasms
Grade 3: transfusion/irrigation required
Grade 4: dialysis required

Casualty Assessment.
For the purpose of this study the treatment plan (preparative regimen followed by allogeneic stem cell transplantation) is defined as the "transplant package"; therefore adverse events known to be caused by components of the transplant package and its direct consequences will be scored as definitive related. Adverse events known to be related to drugs used for the treatment of GVHD and infection episodes will be scored as probable related. When the relationship of the adverse event cannot be ruled out with certainty the AE may be considered possible related. Adverse events known to be related to drugs used for supportive treatment will be scored as unrelated.

The principal investigator will be the final arbiter in determining the casualty assessment.

List of most common expected adverse events.
1. Infections in the presence or absence of neutropenia: fungal, bacterial and or viral infections.
2. Fever: Non-neutropenic or neutropenic without infection
3. Acute graft versus host disease (aGVHD): most commonly manifested by skin rash, diarrhea and abnormal liver function tests could also present with some degree of fever, upper gastrointestinal symptoms (nausea and vomiting) mucositis and eye dryness.
4. Gastrointestinal (GI tract): the GI tract manifestations could be not only due to direct damage from the preparative regiment but also be a manifestation of GVHD or infections. Therefore, the time course and its presentation are crucial when assessing these as adverse events. Nausea/vomiting, mucositis, diarrhea when presented within first 7 to 10 days most likely will be related to the preparative regimen.
5. Skin rash: not related to GVHD could be caused by chemotherapy used for the preparative regimen or antibiotics used a supportive treatment.
6. Transaminitis: liver function test elevation.
7. Pulmonary events: not related to CHF most likely caused by drug injury or infection. These could present with a pneumonitis pattern manifested with shortness of breath, pulmonary infiltrates on chest radiograph, sometimes accompanied by fever and cough and progress to acute respiratory insufficiency and a diffuse bilateral alveolar pattern.
8. Cytokine Storm/ engraftment syndrome: most likely caused by released cytokines.
9. Hemorrhagic cystitis: not related to chemotherapy agents used in the proposed preparative regimen is most likely caused by viral infection.
10. Thrombotic thrombocytopenic purpura (TTP).
11. Veno-occlusive Disease of the Liver (VOD): could be caused by busulfan. Some antimicrobial agents have been also incriminated in its development.
12. Fluid overload due to hydration required for conditioning regimen, blood product transfusions and or IV alimentation
13. Graft failure.

14. Chronic GVHD.
15. For the purpose of this study the following events would not be considered adverse events and would not be recorded in the database:
    1. Flu-like symptoms not associated with infection
    2. Abnormal laboratory findings considered associated to the original disease
    3. Isolated changes in laboratory parameters such as electrolyte, magnesium and metabolic imbalances, uric acid changes, elevations of ALT, AST, LDH and alkaline phosphatase.

Adverse events considered serious.

1. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation).
2. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).
3. Graft Failure/ rejection.
4. Any expected or unexpected event resulting in an irreversible condition and/or leading to death.

Adverse events data collection**.**

From the start of preparative regimen up to D+100 the collection of adverse events will reflect the onset and resolution date and maximum grade; beyond this point some events considered related to chronic GVHD or late complications post-transplant might be recorded only with the first date of their awareness with no grade or resolution date.

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

If a patient is taken off study while an event is still ongoing, this will be followed until resolution unless another therapy is initiated. Pre-existing medical conditions will be recorded only if an exacerbation occurs during the active treatment period. Co-morbid events will not be scored separately.

As stated in the treatment plan, patients treated on this protocol will required supportive care treatment (concurrent medication). These medications are considered standard of care and have no scientific contributions to the protocol; therefore, no data will be captured on the various medications needed or their side effects.

AE and Protocol Deviations Reporting Requirements.

Adverse events will be reported accordingly to MDACC (HSRM chapter 15.001) and SCT&CT Department (HSRM chapter 15.053) policy and procedures. This study will be conducted in compliance however in the event of any protocol deviations or violations these will be reported accordingly to MDACC (HSRM chapter 25).

13.0 Statistical Considerations

13.1 Preliminaries and Objectives

This is an open-label, phase II trial of DCIA followed by immediate AHSCT as therapy for patients with AML that is refractory to high-dose cytarabine. Patients not on this trial would be expected to have an 11% response rate and less than 50% surviving by 3 months with salvage chemotherapy only. The primary objective is to determine the safety and response rate of this new combination with at least 15% of patients alive in remission at 4 months post initial treatment with salvage chemotherapy. Secondary objectives include assessing overall survival (OS), event-free survival (EFS), and early mortality (within first 4 weeks after therapy and within 8 weeks after therapy) for all patients entered on study.

13.2 Endpoints

13.2.1 Primary Endpoints:

13.2.1.1 Overall Response at 4 months post-treatment initiation

Patients will be classified as achieving overall response if they have complete response (CR) or CR without platelet recovery (CRp) or CR with insufficient hematological recovery (CRi) 4 months after initial treatment (i.e., 3 months after planned transplant). Patients who do not have evidence of overall response will be considered not to have overall response, regardless of the reason, including early withdrawals for any reason. Patients without a transplant or who relapse after overall response but before the 4 month will not be considered to have overall response at 4 months post-first treatment.

13.2.1.2 Treatment-Related Mortality within 4 months post-treatment initiation

Patients will be classified as having treatment-related death if they die due to a symptom that is possibly, probably, or definitely related to therapy within 4 months of starting treatment.

13.2.2 Secondary Endpoints

13.2.2.1 Transplant Feasibility

The study will determine the fraction of patients able to receive hematopoietic transplantation.

13.2.2.2 Week and 100 Day Early Treatment Related Mortality (TRM)

The TRM will be recorded at 4 weeks post salvage chemotherapy and at 100 days after AHSCT.

13.2.2.3 Efficacy of DCIA

For each patient, whether or not treatment achieves </=5% bone marrow blasts prior to AHSCT will be recorded.

13.2.2.4 One-year TRM

13.2.2.5 Response/Relapse

Among patients with overall response, the time to relapse will be measured from the date of starting treatment until relapse, progression or last follow-up. Patients who die without relapse will be censored on their date of death. Patients alive, and in overall response, will be censored on their date of last assessment.

13.2.2.6 Overall Survival (OS)
OS is defined as the time from starting treatment until death from any cause. Patients who are alive at the time of analysis will be censored on the date of last contact.

13.2.2.7 Event-Free Survival (EFS)
EFS is defined as the time from starting treatment until disease recurrence, progression, or death, whichever comes first. Patients who are alive and free of progression/relapse will be censored on the last date of evaluation.

13.2.2.8 Safety and Tolerability
Adverse events will be assessed according to NCI CTCAE 4.0. Date of start, date of resolution, grade, and attribution to study drug will be recorded.

13.3 Sample Size Justification
Up to 75 patients will be enrolled with continuous monitoring for safety and efficacy after the 6th patient is enrolled. This sample size ensures that, if the trial continues to 75 patients, a posterior 95% credible interval (CI) of response will be (11.9%, 29.6%), assuming that the response is 0.20 with a beta(0.4, 1.6) prior and 15 responses. We assume 1 patient will be enrolled each month.

13.4 Interim Monitoring
Interim analyses will be carried out continuously after the 6th patient, starting at the desired enrollment of the 7th patient to ensure that patients are exhibiting reasonable toxicity rates and response rates to continue with the trial (see Section 13.4.1). Two Bayesian monitoring designs for delayed response by Cai, Liu, and Yuan[66], one each for toxicity and efficacy will be used to monitor the trial. Stopping rules will be implemented in software provided by the creators, and operating characteristics are based on simulations performed in by their code. Briefly, let π be the proportion of responses. If $\pi \sim \text{Beta}(\zeta,\xi)$, then the posterior distribution for π is

$$f(\pi|y) = Beta(\zeta + \sum_{i=1}^{n} y_i, \xi + \mathrm{n} \sum_{i=1}^{n} y_i)$$

Stopping rules will be based on deciding whether $\Pr(\pi<\phi|y)>\psi$ continuously throughout the trial. Patients who have neither progressed nor been on trial long enough to be evaluable will be specified as having missing data. This method accommodates such missing data by imputing data using a flexible, correlated, piecewise exponential model for the time to response. The method is described more fully in Appendix I. In this protocol we use 6 disjoint intervals for the follow-up time of 4 months.

13.4.1 Interim Stopping Rules Response
For response, the trial will be stopped early if $\Pr(\pi_R < 0.15 \mid \text{data}) > 0.95$, where π_R denotes the overall response rate at 4 months. That is, given the outcomes from the patients who have already been evaluated, and imputed information for those on study without a complete outcome, if it is determined that there is a more than 95% chance that the response rate at 4 months is less than 15%, the trial will be stopped for futility. Once 6 patients are entered onto the trial, information about response and toxic death status will be provided to the statistician prior to enrolling each subsequent patient. The statistician will utilize the Unix-based software to determine whether the trial needs to stop or can continue.

A patient becomes evaluable for response at the following times:

1) Day of 4-month assessment on study
2) Day a decision is made that the patient will not undergo transplant on this study.
3) Day the patient withdraws from study before receiving the 4-month assessment
4) Day of progression or death before 4 months.
5) Is on study more than 6 months but does not undergo assessment

The operating characteristics for efficacy are summarized table 13.4.1 below.

Table 13.4.1 Operating Characteristics for the Trial will Stop if $Pr(\pi R < 0.15 \mid data) > 0.95$

True Response Rate	% Early Termination	Average Sample Size	Total Duration (months)
0.05	94.4%	25.9	13.1
0.10	68.4%	41.3	21.7
0.15	35.6%	56.4	30.5
0.20	14.4%	66.7	36.3
0.25	7.2%	70.6	38.5
0.30	4.2%	72.3	39.5
0.35	2.6%	73.4	40.1

13.4.2 Interim Stopping Rules for Toxicity

Under the model and assumptions described for response above, toxicities will be monitored based on a constant rate of 60%. A trial limiting event (TLE) is any death within 4 months from any cause. The trial will be terminated if Prob(TLE > 0.60 | data) > 0.95. This rule will be implemented in the software as the trial will stop if the 1-Prob(TLE < =0.60| data)>0.95. This is equivalent to ensuring that at least 40% of the patients are alive at 4 months.

The operating characteristics for toxicity are summarized table 13.4.2 below.

Table 13.4.2 Operating Characteristics for Stopping the Trial if Pr(TLE > 0.60 | data) > 0.95

True TLE rate	Early Termination	Average Sample Size	Total Duration (months)
0.4	2.6	73.3	40.1
0.5	6.8	70.7	38.6
0.6	29.0	59.6	32.3
0.7	76.8	38.6	20.1
0.8	99.4	18.2	9.1

13.5 Analysis Plan

Patients' demographic information at baseline will be analyzed, with data summarized in tables listing the number and percentages. Overall response (CR + CRp + CRi) rates and their posterior 95% credible intervals will be estimated using a beta distribution with a prior of Beta (0.4, 1.6). Similarly, the TRM rate at 4 months post treatment initiation will be reported using a prior of Beta (1.2, 0.8). OS, EFS, and relapse rate will be estimated using the Kaplan-Meier method [67]. Similarly, the overall response (CR + CRp + CRi), transplant feasibility, blast recovery, 4 week, 100 day, and 1 year TRM rates, adverse events, and exploratory relationships will be summarized by descriptive tables and figures overall and subsetted by patients entered prior to vs. after the inclusion of venetoclax (Version 10.0).

13.6 Study Accrual and Duration

The maximum number of patients will be 75. The accrual rate will be 1 patient per month. The length of follow-up will be until all patients are evaluable according to section 13.4.1.

14.0 Study Definitions

Engraftment is defined as the evidence of donor derived cells (more than 95%) by chimerism studies in the presence of neutrophil recovery by day 28 post stem cell infusion.

Other definitions used to assess engraftment:

Neutrophil recovery is defined as a sustained absolute neutrophil count (ANC) > 0.5 x 10^9/L for 3 consecutive days.

Engraftment date is the first day of three (3) consecutive days that the ANC exceeds 0.5 x10^9 /L.

Delayed engraftment is defined as the evidence of engraftment beyond day 28 post SC infusion achieved after the administration of therapeutic (high dose) hematopoietic growth factors.

Primary Graft failure is defined as failure to achieve an ANC > 0.5 x 10^9/L for 3 consecutive days by day 28 post SC infusion, with no evidence of donor derived cells by bone marrow chimerism studies and no evidence of persistent or relapsing disease.

Secondary graft failure is defined as a sustained declined of ANC <0.5 x 10^9/L for 3 consecutive days after initial documented engraftment with no evidence of disease progression.

Autologous reconstitution is defined by the presence of ANC > 0.5 x 10^9/L without evidence of donor-derive cells by bone marrow chimerism studies. This can occur at initial engraftment or later after initial engraftment has been documented.

Disease Response for AML

Complete response (CR):
BM < 5% blasts (absence of blasts with Auer rods).
ANC > 1000/ul.
Platelet count >100 x 10e9/ul (independent of red cell transfusions).
Absence of extramedullary disease.

Marrow CR (CRi) (incomplete hematologic recovery):
BM < 5% blasts (absence of blasts with Auer rods).
ANC < 1000/ul or Platelet count <100 x 10e9/ul.
Absence of extramedullary disease.

No Response (NR) or Disease Progression:
BM > 5% leukemia blasts
Persistent presence of blasts in peripheral blood.
Presence of extramedullary disease.

Non-relapse mortality (NRM) is defined as death from any cause other than relapse disease.

15.0 Criteria for Removal from the Study

1. Patient's withdrawal of consent to participate.
2. Serious noncompliance with the protocol treatment which would compromise the study.
3. Graft failure requiring further treatment.
4. Relapse of the malignancy.
5. After two years from transplant in the study.

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