A Phase 2 Study of Clofarabine, Idarubicin, Cytarabine, Vincristine, and Corticosteroids for Patients with Newly Diagnosed or Relapsed Mixed Phenotype Acute Leukemia

I. Objectives

Primary:

To evaluate the response rate of the chemotherapy regimen in patients with mixed phenotype acute leukemia

Secondary:

To evaluate the durability of response, the overall and event-free survival rates, and the safety profile of the regimen

II. Rationale

The diagnosis of acute leukemia is typically based on an evaluation of the morphology, immunophenotype, karyotype, and molecular features of blast cells found in the bone marrow. Most often, the leukemia is clearly assigned to one of the following specific lineages: B-lymphoid, T-lymphoid, or myeloid. Diagnosis is usually unequivocally acute myeloid leukemia (AML), or acute lymphoid leukemia (ALL). ALL is further delineated into B- or T-cell groups. The therapeutic regimen is often designed based on the lineage of the leukemia. Nevertheless, rarely a patient cannot be readily classified due to immunophenotypic evidence of multiple lineages within the cell population [1]. Throughout the years, these leukemias have been referred to by a number of names, including mixed lineage leukemia, biphenotypic leukemia, bilineal leukemia, hybrid leukemia, undifferentiated leukemia, or leukemia of ambiguous lineage. This terminology has been used to describe leukemias with a single population of cells expressing markers from multiple lineages, as well as leukemias exhibiting two distinct blast populations each belonging to a distinct lineage. In 2008, the World Health

Organization (WHO) grouped these processes together under the heading mixed phenotype acute leukemia (MPAL) [2].

The definition of MPAL has evolved over the years, but most now accept the WHO definition published in 2008 [2]. Briefly, a blast population is myeloid if there is sufficient present of myeloperoxidase (MPO) or monocytic differentiation is present. The T lineage is defined by the presence of cytoplasmic or surface CD3. To assign the B lineage, a combination of the expression of several markers is used, including CD19, CD79a, CD22, and CD10.

There is no standard therapy for the group of patients diagnosed with MPAL, and the situation presents a clinical challenge that has not been well studied. Most experience to date comes from the pediatric literature and from older case series. Killick and colleagues identified 25 cases of “biphenotypic” leukemia of 693 cases (3.6%) of acute leukemia over an 8 year period [3]. This cohort included pediatric patients as young as 3 years of age, and adults up to age 46. A wide variety of induction regimens were used, including conventional ALL induction, conventional AML induction, as well regimens that used agents typically used to treat both lineages. Median overall survival (OS) for the 20 de novo patients was 27.3 weeks, which is very poor considering a median age of 25.5 years. Importantly, several patients also had the Philadelphia chromosome, and treatment was before the era of tyrosine kinase inhibitors.

Recently, a group from Saudi Arabia described the clinical characteristics and outcome of 24 pediatric patients diagnosed with MPAL [4]. All patients were treated uniformly with a modified St. Jude Total Therapy-B HR regimen, which that group uses to treat high risk ALL [5]. This regimen involves at least six drugs with activity against lymphoid and myeloid leukemias. All patients were also considered for allogeneic stem cell transplantation (alloSCT) in first complete remission (CR). For patients not undergoing alloSCT, maintenance therapy was continued for 2.5 years. With a median follow up of four years, the survival was 75.7%, which is excellent, but must be interpreted keeping

in mind that the median age was 8.7 years. AlloSCT did not improve the outcome in this small study.

The St. Jude's Children's Research Hospital has recently published their own experience treating pediatric patients with mixed lineage acute leukemias [6]. Thirty-five patients were treated, and the median age was 10. Overall, 23 patients received AML induction (daunorubicin, cytarabine, etoposide), and 12 received standard ALL induction (vincristine, daunorubicin, L-asparaginase, prednisone). Patients receiving AML or ALL induction regimens up front achieved CR rates of 52% and 83%, respectively. Eighty percent of the patients not achieving CR after AML induction subsequently went into CR after ALL induction. The 5-year OS for patients with B/Myeloid or T/Myeloid was 47.8%.

There is very little reported in the literature on adults with MPAL, and as a referral center, we may encounter these patients more often that other institutions. A contemporary regimen employing drugs active against both myeloid and lymphoid leukemias may be the optimal approach. AML is generally treated with anthracyclines combined with cytarabine [7-9]. Recently, the addition of a cladribine, a purine analog, to standard AML induction improved the survival of younger patients with newly diagnosed disease [10]. Clofarabine is also a purine analog that was rationally designed to optimize the pharmacologic shortcomings of two of its predecessors, fludarabine and cladribine [11]. The drug works through several mechanisms including inhibition of deoxyribonucleic acid (DNA) polymerases, inhibition of ribonucleotide reductase, and induction of apoptosis via DNA strand breaks and disruption of mitochondrial integrity [11]. Clofarabine is currently approved for pediatric patients with relapsed and refractory acute lymphoid leukemia after failure of two previous regimens [12].When administered three to six hours prior to cytarabine, in vitro both drugs induce a synergistic effect against AML cells, possibly due to the ability of clofarabine to efficaciously block ribonucleotide reductase [13]. These data have formed the basis for several clinical studies that we and others have conducted in an attempt to exploit this relationship, and hence, improve AML outcomes [14]. Currently, our frontline AML induction regimen of choice is a combination of clofarabine, cytarabine, and idarubicin.

The frontline strategy for the management of adult ALL is similar to pediatrics, and involves induction chemotherapy, multiple rounds of consolidation, a prolonged maintenance phase, as well as central nervous system (CNS) prophylaxis. Most protocols call for approximately 3 years of therapy in total. There are several accepted regimens employed in the United States, and most involve the same key agents which include vincristine, anthracycline (e.g., doxorubicin or daunorubicin), corticosteroids (e.g., prednisone or dexamethasone), with or without some form of L-asparaginase [15-17]. One such regimen is the hyperCVAD, which employs the combination of hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high dose methotrexate and cytarabine [18, 19]. Cycles are repeated approximately monthly for 8 cycles, a point at which patients move to the maintenance portion of the regimen with daily mercaptopurine, monthly vincristine, weekly methotrexate, and monthly pulses of prednisone (POMP). Long term outcomes have been published previously, with five year overall survival (OS) of 38%. Survival was influenced by several prognostic factors that were assessed using multivariable analysis. This is comparable to a number of other regimens that are used depending on center preference.

At the M.D. Anderson Cancer Center (MDACC), MPAL has traditionally been treated with AML induction regimens. It may be optimal to also administer agents that are predominantly used against ALL. Therefore, we are proposing to use a hybrid regimen, with components of both AML and ALL therapy, to treat patients diagnosed with MPAL at MDACC. The backbone of the regimen will be our standard induction for AML, clofarabine, idarubicin, and cytarabine administered at attenuated doses. Importantly, all three of these drugs also have activity against ALL. In addition, we will add vincristine and corticosteroids to the regimen for enhanced lymphoid activity. These are ideal candidates for addition to the regimen for a number of reasons. First, the toxicity profiles do not overlap, allowing us to safely give all five agents during the same course of therapy. Second, corticosteroids are routinely used as a premedication to prevent toxicity induced by moderate to high doses of cytarabine, albeit at lower doses.

III. Background Drug Information

All drugs used in this protocol are available commercially

A. Idarubicin:

Idarubicin is commercially available.

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/m^2; 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%), urticaria
- Gastrointestinal: Nausea, vomiting (30% to 60%); diarrhea (9% to 22%); stomatitis (11%); GI hemorrhage (30%)
- Genitourinary: Discoloration of urine (darker yellow)

B. Cytarabine

Cytarabine is commercially available.

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

• Hematologic: Anemia, Bleeding, Leukopenia, Thrombocytopenia

• Immunologic: Anaphylaxis

• Neurologic: Neuropathy

• Renal: Kidney disease

• Other: Infectious disease, Sepsis

C. Clofarabine

Clofarabine is commercially available.

Mechanism of action:

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:

- Hematologic: Myelosuppression, infections
- Hepato and 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), Steven-Johnson's syndrome, alopecia, conjonctivitis
- Systemic: Fatigue, asthenia, anorexia, lethargy, malaise, mental status changes/coma, alopecia
- Allergic reactions: (including fever, muscle aches, edema, dyspnea)
- Cardiology: Congestive heart failure

- Nephrology: Kidney failure
- Autoimmune reactions: (antiplatelet antibodies, erythema nodosum) and/or chemical imbalances in the blood.

D. Vincristine

Vincristine is commercially available

Mechanism of Action

Binds to tubulin and inhibits microtubule formation; therefore arresting the cell at metaphase by disrupting the formation of the mitotic spindle. It is specific for the S and M phases of the cell cycle.

Adverse Effects

- Peripheral neuropathy
- Constipation
- Ileus
- Loss of deep tendon relexes
- Wrist and foot drop
- Hyponatremia
- Convulsions (rare)
- Severe soft tissue damage if extravasated
- Alopecia
- Paresthesia

E. Dexamethasone and Prednisone

Dexamethasone and prednisone are commercially available

Mechanism of action

Decreases inflammation by suppression of neutrophil migration. In leukemia cells, induces cell cycle arrest and apoptosis.

Adverse Effects

- Salt or fluid retention
- Hypertension
- Muscle weakness or loss of muscle mass
- Osteoporosis
- Aseptic necrosis of the heads of femur or humerus
- Peptic Ulcer
- Pancreatitis
- Skin fragility
- Acute psychosis (rare)
- Elevated blood sugar
- Increased intraocular pressure

F. Methotrexate

Methotrexate is commercially available

Mechanism of Action

Methotrexate is a folate antimetabolite that inhibits DNA synthesis. Methotrexate irreversibly binds to dihydrofolate reductase, inhibiting the formation of reduced folates, and thymidylate synthetase, resulting in inhibition of purine synthesis.

Adverse Effects

- Myelosuppression
- Anorexia

- Mucositis
- Alopecia
- Rash
- Photosensitivity
- Infertility
- Interstitial pneumonitis
- Renal failure
- Hepatic dysfunction
- Leukoencephalopathy
- Menstrual dysfunction

G. 6-Mercaptopurine

6-Mercaptopurine is commercially available

Mechanism of Action

Purine antagonist which inhibits DNA and RNA synthesis; acts as false metabolite and is incorporated into DNA and RNA, eventually inhibiting their synthesis.

Adverse Effects

- Myelosuppression
- Liver failure
- Diarrhea
- Rash
- Nausea and vomiting

H. Rituximab

Rituximab is commercially available

Mechanism of Action

Rituximab is a monoclonal antibody that binds to the CD20 cell surface antigen. Binding of rituximab leads to antibody-dependent cellular cytotoxicity as well as complement mediated cytotoxicity.

Adverse Effects

- Infusion reactions (fever, chills, rigors, hypotension, tachycardia, shortness of breath)
- Reactivation of hepatitis B
- Dermatologic reactions
- Lymphopenia

IV. Eligibility Criteria

Inclusion Criteria

1. Sign an informed consent document

2. Age 18 >/= 18

3. Newly diagnosed or relapsed MPAL, which for this protocol, will be defined as follows:

-Bone marrow result interpreted by the reading Pathologist (or tissue biopsy for cases of extramedullary disease) as: biphenotypic leukemia, bilineal leukemia, undifferentiated leukemia, mixed lineage leukemia, leukemia of ambiguous lineage, T/Myeloid leukemia, B/Myeloid leukemia, or other diagnosis indicating the presence of multiple lineages within the cell population.

Or

-Immunophenotype consistent with WHO definition of MPAL

4. ECOG Performance Status of ≤ 3 at study entry.

5. Adequate organ function as outlined below (unless due to leukemia)

- Serum creatinine ≤ 3 mg/dL
- Total bilirubin ≤ 2.5 mg/dL
- ALT (SGPT) and/or AST (SGOT) ≤ 3 x ULN or ≤ 5 x ULN if related to disease

6. Women of childbearing potential must have a negative serum or urine pregnancy test within 7 days. Women of childbearing potential and men must agree to use contraception at study entry and for the duration of active study treatment.

7. Cardiac ejection fraction ≥ 40% (by either cardiac echo or MUGA scan). Documentation of recent (≤ 6 months from screening) outside reports is acceptable.

8. If newly diagnosed, prior therapy with hydrea and/or steroid and the use of a single or a two day dose of cytarabine (up to 3 g/m^2), for emergency use up to 24 hours prior to start of study therapy is allowed.

Exclusion Criteria

1. Breast feeding females
2. Patients with active, uncontrolled infections
3. Patients with active secondary malignancy will not be eligible unless approved by the Principal Investigator.

V. Treatment Plan

1. Study Design

This will be a phase II, single center, open label trial to assess the safety and efficacy of the regimen outlined below. Patients will be eligible if they are newly diagnosed or with relapsed disease, though these groups will be analyzed separately. We anticipate enrolling 10 – 20 patients per year.

All drugs will be given using commercial supply. For patients over the age of 80 years, further dose decrease beyond what is stated below is allowed at the discretion of the treating physician and if felt in the best interest of the patient.

2. Induction

- Clofarabine 15 mg/m^2 IV daily over approximately 60 minutes for 4 days on days 1 – 4 (for 3 days on days 1-3 for patients 61 – 80 years of age or with performance status (PS) of 2 or 3)
- Idarubicin 10 mg/m^2 IV over approximately 30 – 60 minutes daily for 3 days on days 1 – 3 (for 2 days on days 1-2 for patients 61-80 years of age or with PS 2 or 3)
    - To start 1 – 2 hours after the clofarabine
- Cytarabine 1,000 mg/m^2 IV over approximately 2 hours daily for 4 days on days 1 – 4 (for 3 days on days 1-3 for patients 61 – 80 years of age or with PS 2 or 3)
    - To start 3 – 6 hours after the clofarabine

- Vincristine 2 mg (FLAT dose) IV over approximately 15 – 30 minutes on days 1, 8, and 15
- Dexamethasone 40 mg IV daily for 4 days on days 1 – 4 and days 15 – 18
    - May be given 30 – 60 minutes prior to each dose of cytarabine during induction on days 1 – 4 (if rituximab given, can administer dexamethasone prior to rituximab on day 1)

For patients with CD20 positive disease (≥ 10% of the lymphoid population), rituximab may be added to the regimen as follows:

- Rituximab 375 mg/m^2 IV infused according to package labeling on days 1 and 8.
    - Rituximab will be infused according to the package insert. Premedications will be administered according to departmental standard/prescribing information to prevent infusion related reactions.

The doses and schedule can be modified for the following situations:

- For patients 61 – 80 years of age or with performance status of 2 or 3: give clofarabine and cytarabine for 3 days; give idarubicin for 2 days as mentioned above.
- Further dose reductions or modifications can be considered after discussion with the PI and the discussion documented in the patient's medical record

- All patients will receive at least one lumbar puncture during the first cycle of induction. This is for diagnosis as well as for the application of prophylactic intrathecal chemotherapy (methotrexate 12 mg or cytarabine 100 mg).
- The standard for ALL patients is to administer 8 prophylactic intrathecal chemotherapy treatments during induction and consolidation. Methotrexate is alternated with cytarabine, and patients typically receive two treatments per cycle for the first four cycles.
- For this protocol, giving more than one intrathecal treatment will be at the discretion of the managing physician. This degree of flexibility is warranted due to the heterogeneity of patients with MPAL.

Patients, who have not achieved a complete remission (CR)/complete remission without platelet (CRi) recovery following one induction course can receive a second induction course to optimize response if possible. A second induction course at the same dose as the previous course or in a dose-reduced fashion should not be given until at least 28 days of course 1. If the bone marrow aspirate and/or biopsy(s) performed after the re-induction cycle reveals a remission marrow (CR/CRi), then the patient may proceed with consolidation at the discretion of the treating investigator. In addition, any clinically significant drug-related, non-hematologic toxicity experienced by a patient should return to $\leq$ grade 2 or the baseline grade before the patient continues treatment. Should the patient not have achieved a remission, including CR and CRi after the re-induction course, he/she will be taken off study for failure to respond, unless the patient has

achieved clinical benefit or partial remission, at which time further therapy on protocol may be permitted with approval from the PI.

3. Consolidation

Patients in CR or CRi can continue with up to 6 consolidation cycles.

- Clofarabine 15 mg/m^2 IV daily over approximately 60 minutes for 3 days on days 1 – 3 (for 2 days on days 1-2 in patients 61 – 80 years of age or with PS 2 or 3)
- Idarubicin 6 mg/m^2 IV over 30 – 60 minutes daily for 2 days on days 1 – 2 (3 mg/m^2 for 2 days on days 1-2 in patients 61-80 years of age or with PS 2 or 3)
    - To start 1 – 2 hours after the clofarabine
- Cytarabine 1,000 mg/m^2 IV over approximately 2 hours daily for 3 days on days 1 – 3 (for 2 days on days 1-2 in patients 61 – 80 years of age or with PS 2 or 3)
    - To start 3 – 6 hours after the clofarabine
- Vincristine 2 mg (FLAT dose) IV over 15 – 30 minutes on days 1, 8, and 15
- Dexamethasone 40 mg IV daily for 4 days on days 1 – 4 and days 15 – 18
    - May be given 30 – 60 minutes prior to each dose of cytarabine during consolidation on days 1 – 3 (if rituximab given, can administer dexamethasone prior to rituximab on day 1)

For patients with CD20 positive disease ($\geq$ 10% of the lymphoid population), rituximab may be added to the regimen as follows during consolidation cycles 1 – 3:

- Rituximab 375 mg/m^2 IV infused according to package labeling on days 1 and 8

- Rituximab will be infused according to the package insert. Premedications will be administered according to departmental standard/prescribing information to prevent infusion related reactions.

The doses and schedule can be modified for the following situations:

- For patients 61 – 80 years of age or with performance status of 2 or 3: give clofarabine and cytarabine for 2 days; give idarubicin 3 mg/m^2 for 2 days as mentioned above.

- Further dose reductions can be considered after discussion with the PI

Cycles may be repeated every 3 to 10 weeks based on leukemia response and resolution of clinically significant drug-related toxicities. Prior to each consolidation cycle, the ANC should be $\geq$ 1.0 x 10^9/L, and the platelet count should be $\geq$ 60 x 10^9/L (except for patients who are considered to have achieved a CRp following induction/re-induction and in whom the platelet count may be lower). Patients with borderline values for ANC and platelet count (value up to 10% lower than recommended) can still proceed with the next consolidation cycle if this is judged to be in the best interest of the patients and after discussion with the principal investigator and the discussion documented in the patient's medical record. In addition, any clinically significant drug-related non-hematologic toxicity experienced by the patient must return to $\leq$ grade 2 before receiving the next cycle. Doses missed or held during a cycle of treatment will not be made up and are recorded as being omitted. If patients experience multiple study drug-

related toxicities or experience significant infections, dose adjustments may need to be made based on the most severe toxicity and based on the drug causing the toxicity.

4. Dose Modifications

Drug doses in subsequent consolidation cycles may be modified for clinically significant drug-related > grade 2 non-hematologic toxicities. Dose reductions can also be made in other clinical situations where this step is considered to be in the best interest for the patient and after discussion with the principal investigator and the discussion documented in the patient's medical record. Doses of each individual drug can be modified if a toxicity is considered due this particular drug.

The following are suggestions for dose modifications in subsequent treatment courses:

- Persistent neutropenia (< 1,000) or thrombocytopenia (< 50,000) greater than 42 days from previous cycle: reduce doses of clofarabine, cytarabine and idarubicin by 20 – 25%

- Clinically significant peripheral neuropathy persisting for > 2 weeks:

    - 50% reduction of vincristine (1 mg) for Grade 2 persistent neuropathy

    - Eliminate vincristine for Grade 3-4 neuropathy, seizures and Ileus suspected to be related to vincristine

Organ specific dose adjustments:

	Cytarabine	Idarubicin	Clofarabine	Vincristine
Serum Creatinine (mg/dL)				
2.1 – 3	75% of current dose	75% of current dose	75% of current dose	100% of current dose
Above 3	Discuss with PI			
Bilirubin (mg/dL)				
2 – 3	100% of current dose	75% of current dose	100% of current dose	50% of current dose or omit
3.1 – 5	100% of current dose	50% of current dose	Discuss with PI	Omit
Above 5	100% of current dose	Omit	Discuss with PI	Omit

5. Targeted therapy

Approximately a third of patients with AML may harbor activating FLT3-ITD mutations; such mutations are also observed in patients with biphenotypic or mixed lineage leukemias. We have previously shown that combining sorafenib with chemotherapy in patients with FLT3-ITD mutations is both safe and efficacious. (Ravandi et. al. JCO 2010). Therefore, patients with known FLT3-ITD mutations will be allowed to receive sorafenib therapy at a dose of 400mg orally twice daily on days 1-14 during induction

and then continuously for 28 days per cycle starting with cycle 2, at the discretion of the treating physician. Dose adjustments and interruptions of the sorafenib are per standard of care and at the discretion of the treating physician. These agents will require a separate consent to be completed by the patient and physician. Suggested dose adjustments are in the table below.

Recently, the phase III randomized double-blind RATIFY study was completed and reported for patients with FLT3 mutated AML (Stone RM, et al. NEJM 2017) . 717 patients with FLT3 mutated AML were randomized to receive araC + daunorubicin with or without midostaurin. Midostaurin led to a significant improvement in survival (median 74.7 months for midostaurin vs. 25.6 for placebo; P=0.007). Based on this study, midostaurin was approved for the treatment of patients with FLT3 mutated AML in combination with chemotherapy, the first combination to show a survival benefit in several decades. This has now become part of the standard of care in the treatment of FLT3-mutated AML. Midostaurin has the additional benefit of targeting not only FLT3-ITD mutations, but also point mutations in the FLT3 kinase, such as D835 mutations. Therefore, AML patients with known FLT3-ITD or FLT3 kinase domain mutations will be allowed to receive midostaurin at the recommended dose of 50mg orally twice daily on days 6-19 during induction, and then on days 6-19 during consolidation. Dose adjustments and interruptions of the midostaurin are per standard of care and at the discretion of the treating physician. Suggested dose adjustments are in the table below.

Suggested Sorafenib Dosing Adjustments

Dose Level	Sorafenib Dose and Schedule
0	400 mg PO Twice Daily
-1	400 mg PO Once Daily
-2	200mg PO Once Daily
-3	200mg PO Once Every Other Day

Suggested Midostaurin Dosing Adjustments	
Dose Level	Midostaurin Dose and Schedule
0	50 mg PO Twice Daily
-1	50mg PO Once Daily
-2	50mg PO Every other Day

Other FDA-approved FLT3 inhibitors or targeted therapies (e.g. IDH1 or IDH2 inhibitors) are allowed at the discretion of the treating physician and after discussion with the PI for patients harboring corresponding mutations.

Patients with the presence of the Philadelphia chromosome [translocation (9;22)] or the presence of the bcr-abl fusion gene (detected by PCR or FISH) – such as those with CML myeloid blast phase or 'Philadelphia-positive AML' – may benefit from concomitant therapy with an abl tyrosine kinase inhibitor (TKI). Several orally bioavailable TKIs have now been FDA approved for Philadelphia positive CML and their selection is based on patient tolerance, comorbidities, and abl kinase domain mutations. Patients with bcr-abl positive disease may receive concomitant therapy with an approved TKI, dosed orally,

continuously starting on day 1. The choice and dose will be per the discretion of the treating physician according to standard practice.

6. Central Nervous System (CNS) Prophylaxis and Treatment

All patients will receive at least one lumbar puncture during the first cycle of induction. This is for diagnosis as well as for the application of prophylactic intrathecal chemotherapy (methotrexate 12 mg or cytarabine 100 mg).

The departmental standard of care for ALL patients is to administer 8 prophylactic intrathecal chemotherapy treatments during induction and consolidation. Methotrexate is alternated with cytarabine, and patients typically receive two treatments per cycle for the first four cycles.

For this protocol, giving more than one intrathecal treatment will be at the discretion of the managing physician. This degree of flexibility is warranted due to the heterogeneity of patients with MPAL. This will allow patients with predominantly myeloid disease to be spared unnecessary discomfort and risks. Patients that have predominantly lymphoid disease or others determined to be at high risk for developing CNS disease can receive up to 8 prophylactic intrathecal treatments.

Patients who are diagnosed with CNS disease at any point (cytologically or radiographically) will be managed according to the departmental standard of care with intensive intrathecal chemotherapy treatment.

7. Maintenance Therapy

Maintenance or continuation chemotherapy for up to 3 years is the standard of care for ALL patients. Furthermore, one of the larger experiences in MPAL gave all patients continuation therapy for 2.5 years. Other series have not included maintenance therapy. Therefore, maintenance therapy after the completion of consolidation will be at the discretion of the managing physician. Targeted therapy will continue during maintenance per the managing physician.

Maintenance can be given for up to 30 months as follows:

- Vincristine 2 mg (FLAT dose) IV over 15 – 30 minutes once monthly
- Prednisone 200 mg (FLAT dose) PO daily for 5 days every month
- Methotrexate 20 mg/m^2 PO given once weekly
- 6-Mercaptopurine 50 mg PO three times daily

Doses can be adjusted as clinically warranted at the discretion of the managing physician. In every day clinical practice, doses of methotrexate and 6-mercaptopurine are reduced for myelo- or hepato-toxicity. Vincristine is dose-reduced or omitted for clinically significant peripheral neuropathy. Corticosteroids are dose-reduced or omitted

in patients with active infectious issues. All dose adjustments in maintenance will be captured by research personnel for analysis.

8. Supportive care

Supportive measures such as prophylaxis for tumor lysis syndrome, anti-emetics, erythropoietin, analgesics, blood transfusions, antimicrobials and hematopoietic colony stimulating factors for treatment of cytopenias are permitted. The administration of anti-leukemia therapies is not permitted, except for hydroxyurea which is allowed for up to 7 days per cycle during cycles 1 – 3. The use of up to two doses of cytarabine (up to 3 g/m^2) for emergency use up to 24 hours prior to start of study therapy is also permitted. As stated above, intrathecal chemotherapy is allowed for the prevention and treatment of CNS disease.

Patients may be concurrently enrolling in supportive care clinical trials. Other investigational agents that are used for treatment of other cancers will not be allowed.

Every effort will be made to adhere to the schedule of events and all protocol requirements. Variations in schedule of events and other protocol requirements that do not affect the rights and safety of the patient will not be considered as deviations. Such variations may include laboratory assessments completed outside of schedule, occasional missed required research samples such as PK or PD. All dose adjustments will be made according to the protocol unless otherwise specified.

VI. PRETREATMENT AND POST-TREATMENT EVALUATION

Induction Treatment (Pre-treatment)

1. CBC, differential, and platelet count.
2. Creatinine, bilirubin, ALT (SGPT) and/or AST (SGOT)
3. Echocardiogram or MUGA to assess left ventricular ejection fraction
4. Bone marrow aspirate and/or biopsy with cytogenetics (if bone marrow not done within 30 days or cytogenetics within 90 days. For patients with evidence of leukemia in the peripheral blood, the bone marrow may be omitted after discussion and approval with the principal investigator.)
5. Informed consent
6. Physical exam including vital signs and performance status
7. Concomitant medications and medical history
8. Pregnancy test (serum or urine)

Pretreatment Evaluation

Procedure	Comments	Schedule
Informed Consent	Obtain standard informed consent approved by IRB	Within 14 days of therapy
Medical History	History of present illness, known allergies, prior cancer history as far as traceable, and past medical/ surgical history as far as relevant.	Within 14 days of therapy
Physical Examination	Vital signs (temperature, heart rate, respiratory rate, blood pressure) and performance status.	Within 14 days of therapy
Concomitant Medications	Document concomitant medications	Within 14 days of therapy
Hematology	CBC, differential, and platelet count (the differential may be omitted when the WBC is $\leq$ 500	Within 14 days of therapy

Procedure	Comments	Schedule
Biochemistry	Creatinine, total bilirubin, ALT (SGPT) and/or AST (SGOT)	Within 14 days of therapy
Bone marrow	Aspirate and/or biopsy To confirm complete remission Cytogenetics	Within 30 days of therapy Within 90 days of therapy
Echo or Muga	Assessment of left ventricular ejection fraction	Within 14 days
Pregnancy test	Serum or urine, if female *and* of child-bearing potential only	Within 7 days of therapy

During induction therapy

1. CBC, differential, and platelet count every 3-7 days (the differential may be omitted when the WBC is ≤ 500.). Creatinine, bilirubin, ALT (SGPT) and/or AST (SGOT) once weekly.

2. Physical exam including vital signs (temperature, heart rate, respiratory rate, blood pressure) and performance status. Prior to each treatment course

3. Bone marrow aspirate and/or biopsy on day 28 +/- 7 days to confirm complete remission. Cytogenetics as clinically indicated.

During induction therapy

Procedure	Comments	Schedule
Hematology	CBC, differential and platelet count	Every 3-7 days (the differential may be omitted when WBC is ≤500)
Biochemistry	Creatinine, bilirubin, ALT (SGPT) and/or AST (SGOT)	Once weekly
Physical Examination	Physical exam including vital signs (temperature, heart rate, respiratory rate, blood pressure) and performance status.	Prior to each treatment course

Bone marrow	Aspirate and/or biopsy to confirm complete remission. Cytogenetics as clinically indicated.	On day 28 +/- 7 days

During Post-Remission Therapy (Maintenance and consolidation)

1. Physical exam including vital signs prior to each treatment course
2. CBC, differential, and platelet count prior to each post-CR course
3. Creatinine and bilirubin prior to each post-CR course
4. Bone marrow aspirates as indicated, cytogenetics as clinically indicated.

Long-term Follow-up

Patients will be followed periodically every 6 months (+/- 3 months) for survival via brief phone call, even after being taken off treatment. This may be done over the phone with a member of the study staff. The phone call should last about 10 minutes.

VII. STUDY END POINTS

Primary:

1. Response rate achieved with the chemotherapy regimen

Secondary:

1. Event free survival at 2 years (events defined as death and relapses)
2. Overall survival
3. Remission duration
4. Predictive factors for response and outcome
5. Safety profile

VIII. CRITERIA FOR WITHDRAWAL

Reasons for withdrawal include:

- Withdrawal of consent or the subject refuses to continue treatment and/or procedures/observations.
- Relapse unless the treating physician determines that the patient has achieved clinical benefit, at which time further therapy on protocol may be permitted with approval from the PI.
- Failure to achieve at least a CRi after 2 induction courses

IX. CRITERIA FOR RESPONSE

The response criteria recommended by the NCI and the MDS International Working Group.

Definitions:

Complete Response (CR):

- Neutrophil count ≥ 1.0 $\times 10^9$/L
- Platelet count ≥ 100 $\times 10^9$/L
- Bone marrow aspirate $\leq$ 5% blasts
- No extramedullary leukemia

CRi:

- Response as in CR but platelets <100 $\times 10^9$/L

Partial response (PR):

- Neutrophil count ≥ 1.0 $\times 10^9$/L
- Platelet count ≥ 100 $\times 10^9$/L
- ≥ 50% reduction in bone marrow blasts over baseline

Clinical benefit:

In addition to IWG criteria, in AML, a decrease in bone marrow blasts to <5% will also be considered clinical benefit.

Stable Disease:

In addition to IWG criteria and in absence of any of the above response criteria, patients will be considered to have stable disease if the bone marrow blast percent does not increase compared to pretreatment level.

Relapse:

Increase of bone marrow blasts to > 10% after an initial response.

X. REPORTING REQUIREMENTS

All adverse and serious adverse events will be recorded and reported according to the Department of Leukemia guidelines (appendix C)

XI. STATISTICAL CONSIDERATIONS

This is a Phase II study of clofarabine, idarubicin, cytarabine, vincristine, and corticosteroids for patients with newly diagnosed or relapsed/refractory mixed phenotype acute leukemia

The primary objective is to evaluate the efficacy, with primary endpoint of response (CR or CRi) during a 2 month window. The two groups of patients will be evaluated separately; however accrual to both groups will be concurrent.

Part I: Newly diagnosed patients

A maximum of 40 newly diagnosed will be enrolled. The toxicity, 4-week mortality rate, and efficacy (response) will be monitored during the study, and all the data will be used to update the prior distributions for toxicity and efficacy parameters. The study will be stopped for toxicity, 4-week mortality and futility based on the following stopping rules.

1.1 Response

The historical data suggested that the response rate in the standard treatment for the study patient is about 50%, it is anticipated that with the study treatment the response rate will increase to 70%.

Response will be monitored closely in all patients using the method of Thall et al (20). The interim monitoring will begin after 10 patients have been enrolled to the study, and then by a cohort of 5 patients. Denote the probabilities of response for the standard treatment and this experimental study by P_S and P_E, respectively We assume $P_S \sim$ beta(50, 50) to reflect the historical response rate and $P_E \sim$ beta (1, 1), which have the

same response rate but a bigger various. We will stop the trial if at any point Pr (P_E > P_S + 0.2 | data) < 0.01. That is, we will stop the trial if, at any time during the study, we determine that there is less than 1% chance that the response rate in the study is 20% more than that of the standard treatment. Stopping boundaries corresponding to this stopping rule and operating characteristics are listed in table 1a and 1b respectively. For example, if 3 or fewer patients have response in the first 10 patients, the trial will be stopped.

If the study is not stopped early and all 40 patients have been enrolled to the study and evaluated, and assuming that 28 out of the 40 patients have response to the treatment, then the 95% credible interval for the response rate will be (0.54, 0.82).

Table 1a: The trial will be monitored according to the following stopping boundaries for CR/CRi.

Number of Patients Evaluated	Recommend Stopping if Response Observed in n or less patients
10	3
15	5
20	8
25	11
30	13
35	16

Table 1b: Operating characteristics for response monitoring

True CR Probability	Probability of Early Stop	Achieved Sample Size Percentile (10 25 50 75 90)
0.3	0.99	10 10 10 15 20
0.4	0.861	10 10 20 25 40
0.5	0.487	10 20 40 40 40
0.6	0.137	25 40 40 40 40
0.7	0.018	40 40 40 40 40
0.8	0.0009	40 40 40 40 40
0.9	<0.0001	40 40 40 40 40

1.2 4-week mortality

Mortality in the first 4 weeks will be monitored closely in patients using the method of Thall et al (1995). Denote the probability of death within 4 weeks by D_E. We assume D_E ~ beta (0.2, 1.8). We will stop the trial if at any point $Pr(D_E > 0.1 \mid data) > 0.90$. That is, we will stop the trial if, at any time during the study, we determine that there is more than 95% chance that the mortality rate is more than 10%. The trial will be stopped if (the number of death in the 1st 4 weeks observed) / (among the number of patients) ≥ 3/10, 4/12, 5/19, 6/26 and 7/33. The operating characteristics are listed in table 2.

Table 2: The operating characteristics for mortality monitoring are summarized in the following table

True probability of death	Probability of early stop	Sample size percentiles (10, 25, 50, 75, 90)
0.01	<0.0001	40 40 40 40 40

0.05	0.027	40 40 40 40 40
0.1	0.191	16 40 40 40 40
0.15	0.509	10 15 39 40 40
0.2	0.782	10 10 17 37 40
0.25	0.924	10 10 11 22 36

1.3 Toxicity

Toxicity will be monitored closely in all patients using the method of Thall et al (1995). Denote the probability of toxicity by θ_E, where toxicity is defined as any Grade 3 or greater clinically significant non-hematological toxicities related to the treatment. We assume θ_E ~ beta (0.6, 1.4). We will stop the trial if we $Pr(\theta_E > 0.30 \mid data) > 0.92$. That is, we will stop the trial if, at any time during the study, we determine that there is more than 95% chance that the toxicity rate is more than 30%. Stopping boundaries corresponding to this stopping rule and operating characteristics are listed in table 3a and 3b respectively.

Table 3a: The trial will be stopped according to the following boundaries for toxicities

Number of Patients Evaluated	Recommend Stopping if Toxicity Observed in n or more patients
10	6
15	8

20	10
25	12
30	14
35	16

Table 3b: The operating characteristics for toxicity monitoring are summarized in the following table

True toxicity probability	Probability of early stop	Sample size percentiles (10, 25, 50, 75, 90)
0.1	0.0001	40 40 40 40 40
0.2	0.011	40 40 40 40 40
0.3	0.112	30 40 40 40 40
0.4	0.446	10 15 40 40 40
0.5	0.833	10 10 15 30 40
0.6	0.981	10 10 10 15 25

Analysis Plan:

For discrete or categorical data, descriptive statistics will include tabulations of frequencies. For continuous data, summary statistics including n, mean, standard deviation, median, minimum and maximum will be computed. The posterior response rate their 95% credible intervals will be estimated.

Part II: Relapsed patients

Up to 20 relapsed/refractory patients who have no other treatment options will be enrolled in the study. The descriptive analysis will be used to summarize the response data, and the Kaplan-Meier method will be applied to estimate the median survival and 1-year survival rate. The study will monitor the 4-week mortality and toxicity use the method of Thall et al (1995).

2.1. 4-week mortality

Mortality in the first 4 weeks will be monitored closely in patients using the method of Thall et al (1995). Denote the probability of death within 4 weeks by D_E. We assume D_E ~ beta (0.2, 1.8). We will stop the trial if at any point $Pr(D_E > 0.1 \mid data) > 0.8$. That is, we will stop the trial if, at any time during the study, we determine that there is more than 80% chance that the mortality rate is more than 10%. The trial will be stopped if (the number of death in the 1st 4 weeks observed) / (among the number of patients) ≥ 3/10, 4/17. The operating characteristics are listed in table 4.

Table 4: The operating characteristics for mortality monitoring are summarized in the following table

True probability of death	Probability of early stop	Sample size percentiles (10, 25, 50, 75, 90)
0.01	0.001	20 20 20 20 20
0.05	0.045	20 20 20 20 20
0.1	0.218	12 20 20 20 20
0.15	0.453	10 12 20 20 20

0.2	0.67	10 10 14 20 20
0.25	0.826	10 10 11 15 20

2.2 Toxicity

Toxicity will be monitored closely in refractory/relapsed patients using the method of Thall et al (1995). Denote the probability of toxicity by θ_E, where toxicity is defined as any clinically significant Grade 3 or greater toxicities related to the treatment. We assume θ_E ~ beta (0.6, 1.4). We will stop the trail if we Pr(θ_E > 0.30 | data) >0.8. That is, we will stop the trial if, at any time during the study, we determine that there is more than 80% chance that the toxicity rate is more than 30%. The trial will be stopped if (the number of patients with toxicity observed) / (among the number of patients) ≥ 5/10, 6/12, 7/15 and 8/18. The operating characteristics are listed in table 5.

Table 5: The operating characteristics for toxicity monitoring are summarized in the following table

True toxicity probability	Probability of early stop	Sample size percentiles (10, 25, 50, 75, 90)
0.1	0.002	20 20 20 20 20
0.2	0.071	20 20 20 20 20
0.3	0.315	10 14 20 20 20
0.4	0.65	10 10 13 20 20
0.5	0.888	10 10 10 13 20

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