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Protocol Title	Phase I/II Study of the Combination of Quizartinib (AC220) with 5-Azacytidine or Low-Dose Cytarabine for the Treatment of Patients with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)
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Phase I/II Study of the Combination of Quizartinib (AC220) with 5-Azacytidine or Low-Dose Cytarabine for the Treatment of Patients with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)

1.0 OBJECTIVES

1.1 Primary Objectives

1.1.1 Phase I: To determine the DLT and MTD of the combination of quizartinib (AC220) with either 5-azacitidine (AZA) or low-dose cytarabine (LDAC) in patients with acute myeloid leukemia (AML) or high-risk myelodysplastic syndrome (MDS).

1.1.2 Phase II: To determine the clinical activity of the combination of quizartinib with either AZA or LDAC in patients with AML or MDS.

1.2 Secondary Objectives:

1.2.1 Phase I: To determine the clinical activity of the combination of quizartinib with either AZA or LDAC in patients with AML or MDS.

1.2.2 Phase II: To determine the safety of the combination of quizartinib with either AZA or LDAC in patients with AML or MDS.

1.2.3 Both:

1.2.3.1 To determine the induction of hypomethylation, DNA damage and FLT3 signaling during therapy with this combination and its correlation with response.

1.2.3.2 To determine the effect of this combination therapy on plasma levels of FLT3-ligand

1.2.3.3 To determine the pharnacodynamicvs of this combination therapy in patients with AML or high-risk MDS.

2.0 BACKGROUND

Acute myeloid leukemia (AML) is a malignancy of immature granulocytes or monocytes. Malignancy is characterized by accumulation of leukemic blastocytes and blockade of normal bone marrow production resulting in thrombocytopenia, anemia, and neutropenia. In the U.S., approximately 11,930 new cases of AML are expected to be diagnosed in 2006, with an estimated 9,040 deaths occurring in the same time period. Almost all newly diagnosed cases, as well as deaths, will be in adults.[1] 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. 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 to be directly related to age.[2]

Signaling via receptor tyrosine kinases (RTKs) is frequently dysregulated in disease. FLT3 (FMS-like tyrosine kinase III) is a transmembrane tyrosine kinase that belongs to the Class III family of RTKs. FLT3 is primarily expressed on immature hematopoietic progenitors and also on some mature myeloid and lymphoid cells.[3-5] FLT3 is activated following binding of FLT3 ligand, which causes receptor dimerization leading to increased kinase activity and activation of downstream signaling pathways including Stat5, Ras, and PI3'kinase.[6-8] FLT3 plays a normal role in the regulation of survival and proliferation of hematopoietic progenitor cells, in particular by synergy with other RTKs and cytokine receptors.[9-11] FLT3 is also expressed in acute myeloid leukemia cells from approximately 90% of patients and stimulates survival and proliferation of leukemic blasts.[12-14]

Additionally, FLT3 is mutated in 30% of AML cases.[15] The two leading types of mutations found in AML include internal tandem duplications in the juxtamembrane domain (ITD, 17-34%) and mutations in the activation loop (approximately 7%). Patients with mutations in FLT3 have a worse prognosis when treated with conventional chemotherapy compared to patients with wild-type FLT3.[15] Initial studies using small molecule FLT3 inhibitors have offered encouragement that when added to the conventional arsenal of AML treatments, patients with AML expressing mutated FLT3 may experience significant clinical benefit.[15]

2.1 AC220 (Quizartinib)

AC220 is a novel second-generation Class III RTK inhibitor with potent and highly efficacious FLT3 activity in vitro and in vivo. Activating gene mutations in the RTK FLT3 (FLT3 mutant) are present in approximately 30% of patients with AML. These patients with FLT3-mutants have a significantly worse prognosis than patients with FLT3 wild type (FLT3 WT), suggesting that the activated kinase is a driver of AML and a potential target for kinase inhibitor therapy.

Toxicology studies with AC220 in rats, dogs, and monkeys up to 90 days in duration have shown that the principle toxicological target organs were the bone marrow and lymphoid organs in rat, dog, and monkey. These findings are consistent with the presumed FLT-3 and c-KIT kinase inhibition mechanism of action of the drug. In addition, toxicological effects were observed in the kidney (all species), the liver (dog only) and the ovary, vagina, and testes (rat only). Target-organ toxicity appears to be dose-, time-, and species-dependent, but reversible following a 28-day reversal period, except for the dog-specific liver toxicity. Bioavailability and exposure were good across species, ranging from 16% in monkeys to 40% in dogs. Plasma protein binding with AC220 was high (> 99%) in all 5 species examined (mouse, rat, dog, monkey, and human) and the compound has been shown to penetrate the brain poorly. In vitro CYP450 studies demonstrate that AC220 is neither an inhibitor nor inducer of major human CYP isoforms. Evidence

from rat toxicokinetic studies indicate that AC220 does not accumulate nor does it induce its own metabolism. A major pharmacologically active metabolite, termed AC886, has been identified in the plasma of rat, dog, monkey, and humans.

A cardiovascular safety pharmacology study in cynomolgus monkeys demonstrates that single oral doses of AC220 result in prolonged QTc interval at ≥10 mg/kg doses and biologically significant increased systolic blood pressure at ≥30 mg/kg doses. It should be noted that increases in blood pressure were not observed in the Phase 1 dose-escalation study, Protocol AC220-CP0001; henceforth called Study CP0001.[16] No QTc interval prolongation was evident in monkeys dosed with 3 mg/kg AC220. The prolonged QTc interval is presumably not related to inhibition of the I_{Kr} ion channel as evidenced by negative human ether-a-go-go-related gene (hERG) assays conducted for AC220 and the major metabolite, AC886 (11%-15% I_{Kr} channel inhibition at 10 μM for AC220 and < 10% at 10 μM for AC886). There were no apparent electrocardiogram (ECG) abnormalities in the dog (28- and 90-day) or monkey (28-day) general toxicology studies. In addition, there were no apparent toxicologically relevant AC220-related heart microscopic changes in the rat, dog, or monkey general toxicology studies. At steady-state, there is approximately a 1.5-fold exposure margin of AC220+AC886 between monkeys dosed with 30 mg/kg and humans dosed with 200 mg/day.

Interim results from Study CP0001[16] have shown that AC220 has been well tolerated in the 76 patients treated thus far. The majority of adverse events (AEs) observed in the study were those associated with the underlying disease. In addition, the safety profile was similar between patients receiving continuous dosing (28 days continuous dosing as 1 cycle) and those on the intermittent schedule (14 days dosing and 14 days rest as 1 cycle). In spite of limitations of the Phase 1 design, e.g., limited evaluation of response and the potentially suboptimal intermittent dosing schedule, encouraging preliminary evidence of clinical activity has been observed in the first 76 patients treated with AC220. The overall response (complete remission [CR] + partial remission [PR]) observed in all AC220-treated patients was 32% (24/76). Responses were defined per modified Cheson criteria.[17] Ten patients achieved a CR defined as a decrease of < 5% blast in bone marrow: 2 complete hematological recovery, 4 with incomplete platelet recovery (CRp), and 4 with incomplete platelet and neutrophil recovery (CRi). One of these patients also had complete resolution of leukemia cutis. Fourteen patients had PR, defined as a decrease of ≥ 50% blasts to levels of 5% to 25% in the bone marrow but with incomplete peripheral recovery of neutrophils and platelets. Most of the patients had best bone marrow responses (14/22, 64%) during the first 28 days of treatment (defined as Cycle 1). Median duration of response was 14.1 weeks (range, 4 to 61+ weeks). Consistent with the proposed mechanism of action, the response in the FLT3-ITD mutant population was the highest with an overall response rate of 61% (11/18) including 6 PR, 3 CRi, 1 CRp, and 1 CR.

The dose-limiting toxicity (DLT) as per protocol in Study CP0001 was National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3 (v3) Grade 3 QTcF (corrected QTc by Fridericia's correction factor) interval prolongation at the 300-mg dose of AC220 administered continuously, and considered to be possibly drug related. It should be noted that this study did not exclude high risk cardiac patients, including those with abnormal baseline QTcF prolongation, nor did it exclude the administration of QT/QTc-prolonging concomitant medications, and patients were sometimes in a state of electrolyte imbalance with low serum potassium and/or magnesium levels. These patients with Grade 3 QTcF interval prolongation have been asymptomatic, with no evidence of arrhythmias and have had reversal of QTcF prolongation when AC220 dosing was interrupted. The ECGs were originally analyzed by the sites, however they were subsequently re-read using a centralized digital analysis system employed by a central ECG laboratory (EResearch Technology in Philadelphia, PA) and a formal analysis was performed. This determined that the ECG data in Study CP0001 did not demonstrate any clear signal of any clinically important effect on heart rate, PR (atrioventricular conduction), or QRS interval duration (depolarization) or morphology. The primary finding in this first-in-human study with limited cardiac safety data was that AC220 appears to have a marked effect on cardiac repolarization, which is best defined by the Fridericia's corrected QT data (QTcF). The time-averaged mean change from baseline for QTcF duration for the intermittent dose groups showed no QTcF effect for the 12- to 60-mg dose groups. Starting with the 90-mg dose, however, the QTcF interval change from baseline was > 20 ms and tended to increase with dose, up to 30 to 38 ms for the 300- to 450-mg doses. For the continuous treatment dose groups, the QTcF interval change from baseline for 200 mg was 26 ms and for 300 mg was 54 ms. During the first 14 days of dosing, the intermittent and continuous treatment dose groups at 200 mg and 300 mg experienced the same exposure to AC220, and therefore, the data over this time period may be considered together. Taken together, the QTcF interval change from baseline for the 200-mg dose was 22 ms to 26 ms, and for the 300-mg dose was 38 ms to 54 ms, and the incidence of new cases (not seen at baseline) of Grade 3 QTcF interval prolongation (> 500 ms) was 9.5% (2/21 patients) at 200 mg and 30% (3/10 patients) at 300 mg. This demonstrates a clear dose-related and marked QTcF interval change at the ≥200-mg dose level. The QTcB (Bazett's correction factor) results were comparable. The onset of increase to > 500 ms QTcF occurred as early as 2 hours after the first dose and by Cycle 1 Day 8 of dosing in the cases that were observed. Additional observations were made in women and patients of > 62 years, as is usual in these settings. The pharmacokinetic-pharmacodynamic (PK-PD) relationship for parent and metabolite was also indicative that AC220 caused a positive QTc response of a magnitude that was observed by the ECG data.

2.2 5-azacytidine

5-azacytidine, an analog of the pyrimidine nucleoside cytidine, has effects on cell differentiation, gene expression, and deoxyribonucleic acid (DNA) synthesis and metabolism. Since the early 1970s, 5-azacytidine has been investigated primarily in the US for the treatment of acute leukemia. Clinical studies have focused mainly on patients with disease refractory to conventional chemotherapy. Results of these investigations demonstrated activity of 5-azacytidine in the treatment of AML. Clinical studies subsequently evaluated the effects of 5-azacytidine in a variety of other malignant and hematologic disorders, including solid tumors, hemoglobinopathies (eg, thalassemia and sickle cell anemia), and MDS. In 1984, the Cancer and Leukemia Group B (CALGB) began a series of clinical studies with 5-azacytidine in patients with MDS. These studies, in addition to other supportive data, led to the approval of Vidaza® (5-azacytidine) in May 2004 for the treatment of MDS.

5-azacytidine inhibits the methylation of newly synthesized DNA by inhibiting DNA methyltransferase (DNMT).[18-20] Increased methylation of DNA (hypermethylation) may result in the silencing of critical genes responsible for cell growth control and differentiation. Hypermethylation of CpG islands spanning the promoter regions of tumor suppressor genes is commonly associated with cancers.[21] It is now widely recognized that hypermethylation of DNA is frequently associated with myelodysplastic syndromes and other cancers,[22-24] such as renal,[25] melanoma,[26] breast,[27] colorectal,[28] non-small cell lung[29] and hematologic malignancies.[30] 5-azacytidine is believed to exert its antineoplastic effects through hypomethylation and cytotoxicity on abnormal hematopoietic cells in the bone marrow.[31-35] Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation.[21,36,37] The cytotoxic effects of 5-azacytidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms.[31,38-40]

The cytotoxicity of 5-azacytidine is proportional to dose and exposure time.[31,32] Although the mechanisms of cytotoxicity are complex and multifaceted, there is general agreement that incorporation of 5-azacytidine into DNA and ribonucleic acid (RNA), and inhibition of protein synthesis, are critically important.[41] Cytotoxicity is greatest in cells that are proliferating (S phase) and metabolically active.[31] Cytotoxic effects may also be mediated through induction of the DNA damage response pathways.[40] Nonproliferating cells are relatively insensitive to 5-azacytidine.[31]

Toxicology studies have been conducted in mice, rats, dogs, and Rhesus monkeys.[42] Most of the studies were performed during the 1970s and early 1980s according to existing guidelines and standards in place during that period. The preclinical studies identified the bone marrow, liver, kidneys, and lymphoid tissues (spleen, lymph nodes, and thymus) as the main target organs of toxicity for 5-azacytidine.[42] In single-dose studies, the lethal dose of 5-azacytidine after intravenous (IV) administration in mice, rats, and dogs was approximately 250

mg/m^2. Repeated daily dosing appears to increase the toxicity of 5-azacytidine.[42] The genotoxicity of 5-azacytidine is consistent with that of other nucleoside analogs that interact with nucleic acids.[42] Likewise, similar to other agents with cytostatic properties, 5-azacytidine was embryotoxic and reduced the reproductive performance in mice and rats.[42]

Limited 5-azacytidine pharmacokinetic data are currently available. Based on human plasma concentrations of total radioactivity (which represents parent drug plus circulating metabolites), 5-azacytidine is rapidly absorbed when given subcutaneously (SC), with maximum plasma concentrations found 0.5 to 2 hours after dosing.[42] 5-azacytidine and/or its by-products are then rapidly cleared by the kidneys. The half-lives and percent radioactivity recovered in urine are similar following IV and SC routes of administration. The effects of renal or hepatic impairment, gender, age, or race on the pharmacokinetics of 5-azacytidine have not been studied.[42] A single dose (75 mg/m^2) SC versus IV crossover study in 6 MDS subjects[43] revealed an approximate bioavailability of 89% for the SC dose (range 52% to 128%) with mean half-lives of 0.69 hour and 0.36 hour after SC and IV administration, respectively. These results demonstrated that 5-azacytidine is rapidly and nearly completely absorbed after SC administration and that elimination is also rapid. The apparent SC clearance (167 L/h or 2791 mL/min) and systemic IV clearance (147 L/h) of 5-azacytidine exceeded the glomerular filtration rate (approximately 125 mL/min) and total renal blood flow (1200 mL/min) in healthy subjects. This indicates that non-renal elimination (eg, metabolism, hydrolysis, and/or degradation) plays a role in the elimination of parent 5-azacytidine. In addition, 5-azacytidine 75 mg/m^2 was generally well-tolerated after single SC injection or IV infusion over 10 minutes.[43]

A number of studies have looked at different parenteral doses and schedules of 5-azacytidine, finding maximum tolerated doses of up to 500 mg/m^2 when administered weekly.[44]

During the two decades between the start of the CALGB studies and the approval of 5-azacytidine, a new understanding of MDS has developed, such as the World Health Organization (WHO) diagnostic criteria, the International Prognostic Scoring System (IPSS), and the International Working Group (IWG) response criteria. Silverman et al. reevaluated the combined data (N = 309) from 3 of the CALGB studies using the WHO classification system for MDS and AML and the IWG response criteria.[45] Using the IWG response criteria in MDS patients, response rates were between 40% and 70% in 5-azacytidine treated patients. Ten to 17% of patients achieved a complete remission; partial remission was rare; and 23% to 36% of patients had a hematologic improvement. In patients with AML (N = 103), 35% to 48% had hematologic improvement or better responses. The median survival time for 27 patients assigned to 5-azacytidine was 19.3 months compared with 12.9 months for the 25 patients assigned to observation.[45]

A randomized international Phase III trial (Study 5-azacytidine PH GL 2003 CL 001) for higher-risk MDS patients, classified by FAB as RAEB, RAEB-T, or CMML with 0-29% marrow blasts, with an IPSS of Intermediate -2 or High by central pathology/cytogenetic review was recently reported.[46] Patients were randomized to 5-azacytidine (75 mg/m^2/day x 7days in 28 day cycles) or conventional care regimens (CCR) , where CCR was pre-selected by the Investigator as best supportive care (transfusions, antibiotics, and G-CSF for neutropenic infection), low-dose cytarabine (20 mg/m^2/day x 14 days in 28 day cycles); or standard chemotherapy (conventional induction/consolidation). Patients were stratified by FAB/IPSS and randomized 1:1 to 5-azacytidine or CCR. This trial did not allow erythropoietin. Three-hundred fifty eight patients (70% male) were randomized at 79 centers to 5-azacytidine (N=179) or CCR (N=179): best supportive care only (N=105, 59%), low-dose cytarabine (N=49, 27%), or standard chemotherapy (N=25, 14%). Median age was 69 years (range 38-88 years). The 5-azacytidine and CCR groups were comparable for baseline patient characteristics. At baseline, 95% of patients were higher risk: RAEB (58%), RAEB-T/WHO AML (34%), CMML (3%), and other (5%). By IPSS, 87% were higher risk: Intermediate -2 (40%), High (47%), and 13% Indeterminate/other. 5-azacytidine was administered for a median of 9 cycles; low-dose cytarabine for 4 cycles. Median follow-up for the survival analysis was 21.1 months. 5-azacytidine demonstrated statistically superior overall survival compared to CCR, with a median overall survival of 24.4 months vs. 15 months for CCR (stratified log-rank p=0.0001, hazard ration 0.58). Two-year survival approximately doubled in the 5-azacytidine arm compared to CCR: 51% vs. 26% (p<0.0001). 5-azacytidine was well tolerated with safety data consistent with previous reports.

Further details can be found in the 5-azacytidine Investigator's Brochure, which contains comprehensive pharmacology, toxicology, pharmacokinetics, pharmacodynamics, metabolism, preclinical, and clinical efficacy and safety data information.[42]

2.3 Cytarabine

Cytarabine has been the mainstay of AML therapy for nearly 40 years. In young patients cytarabine is usually combined with an anthracycline. With this standard therapy the CR rates are 60% to 70% and the cure rates are 15% to 35%. Prognosis is related to 1) leukemia karyotype, 2) patient age, and 3) performance and organ functions. Patients with t(8;21), inversion 16 or t(15;17) have CR rates of 90% and cure rates of 50% to 80%. Younger patients (age $\leq$ 50 years) with diploid karyotypes have CR rates of 70% to 80% and cure rates of 20% to 25%. Older patients and those with adverse karyotypes have CR rates of 35% to 50% and cure rates of 10% or less. Older patients with AML have a poor prognosis. This is due in part to the poor tolerance to therapy, and also the higher frequency of poor prognostic features, such as high-risk cytogenetic abnormalities and MDR expression. The experience at M.D. Anderson from 1996 to 2000 for patients age

65 years or older is a clear example of this poor prognosis. We treated 245 patients in this age group during this period. CR was achieved in 118 (48%) while induction mortality (i.e., death within 7 weeks from the start of chemotherapy) occurred in 54 (22%). 38 patients (18%) were alive in CR after year, and 16 (8%) at 2 years. Low-dose cytarabine is better tolerated and has been shown to improve survival compared to supportive care ± hydroxyurea,[47] and has become a "de facto" standard therapy for older patients not fit for intensive chemotherapy. However, the median survival is still only approximately 4 months.[47] Thus, there is a need to improve the results obtained with standard chemotherapy.

Rationale for study:
We hypothesize that adding AC220 to a hypomethylating agent such as AZA or to standard chemotherapy such as cytarabine may improve the response rate expected from the use of either agent alone. In the first portion of this study, we propose to explore the proper dose for these combinations and will explore this in patients with refractory or relapsed AML or MDS. Once the dose finding study is completed we will take this approach to a frontline or first salvage setting. Furthermore, in the phase 2 study of AC220 as single agent in patients with refractory or relapsed AML without FLT3 mutations, a response rate of 30% to 36% was reported in older patients receiving first salvage, or patients of any age receiving second salvage. This is a very significant response rate achieved with a single agent, well-tolerated agent, not regularly achieved with other standard or investigational agents Despite a high response rate for this setting with a single-agent oral agent, the use of AC220 in this subset of patients has not been further explored clinically. We hypothesize that the combination of AC220 and azacytidine may result in a higher response rate and may offer a valuable salvage strategy to this patient population.

2.4 Recently, the results of a randomized trial comparing two lower doses of quizartinib were reported. In this trial, patients with relapsed or refractory AML with FLT3 mutation were randomized to receive quizartinib 30 mg or 60 mg orally, daily. A total of 76 patients were enrolled (38 to each arm). The overall composite response rate (CRc, which was the primary endpoint) was identical for the two arms (47%). The overall response rate was slightly higher with 60 mg daily (71%) than with 30 mg daily (61%) because of a higher rate of partial responses in the 60mg arm (24%) compared to the 30 mg arm (13%). QTc prolongation to >500 msec (the dose limiti98ng toxicity for quizartinib) occurred in only 3% with 60 mg and 5% with 30 mg. When comparing these results with a prior study exploring higher doses (90 mg, 135 mg, and 200 mg) it is clear that the rate of CRc is identical at all dose levels with some increase in PR with progressively higher doses. However, QTc prolongation is clearly dose dependent and prolongations >60 msec from baseline occur in 40% or more of the patient s treated at doses of 90mg or above, exceeding the threshold of acceptable grade 3 toxicity for the standard definition of MTD. Although these prolongations have been asymptomatic and transient, resolving with lower doses, based on this new data we have decided not to explore a dose level of 90 mg daily and expand 60

mg (i.e., the starting dose level) for the phase 2 portion of the study provided MTD is not exceeded as defined in the protocol section 5.2.5. If MTD is exceeded at this dose level, 30 mg will be explored.

	2689-CL-2004			AC220-002 (Cohort 2)		
	30 mg/day (N = 38)	60 mg/day (N = 38)		90 mg/day (N=57)	135 mg/day (N=67)	200 mg/day (N=12)
Best Response						
CRc Rate	47%	47%		47%	45%	42%
PR Rate	13%	24%		25%	28%	50%
Maximum change in QTcF from baseline (msec)						
≤ 30	50%	44%		9%	9%	0%
> 30 to ≤ 60	47%	36%		46%	51%	8%
> 60	3%	19%		46%	39%	92%

3.0 STUDY DESIGN

- This will be a phase I/II, two-arm, open-label study.
- Patients will receive therapy with quizartinib orally daily on 28 day cycles.
- In addition patients will receive either AZA subcutaneously or intravenously daily for the first 7 days of each treatment cycle or LDAC subcutaneously twice daily for the first 10 days of each treatment cycle. Cycles will be repeated approximately every 28 days, and therapy will be continued until clinically significant disease progression or documentation of unacceptable toxicity.

4.0 PATIENT SELECTION

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied, must be reviewed by the Principal Investigator or his/her designee prior to enrollment of that patient. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to initiating treatment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.1 Inclusion Criteria

4.1.1 Patients with MDS, CMML or AML

4.1.2 For Phase I only

4.1.2.1 Refractory or relapsed disease defined as follows:

4.1.2.1.1 Patients with MDS or CMML should have failed prior therapy (e.g., with a hypomethylating agent, clofarabine, and/or with lenalidomide).

4.1.2.1.2 Patients with AML should have failed any prior induction therapy or have relapsed after prior therapy.

4.1.2.1.3 Patients (any age) with MDS or CMML who received therapy with a hypomethylating agent and progress to AML are eligible at the time of diagnosis of AML regardless any prior therapy for AML. The WHO classification will be used for AML.

4.1.2.1.4 Patients with any of the eligible diagnoses who have received no prior therapy are eligible if not candidates to receive standard intensive therapy (ie, high-dose cytarabine-based chemotherapy).

4.1.2.2 Patients are eligible regardless of their FLT3 mutation status

4.1.2.3 Age ≥18 years

4.1.3 For Phase II only: two cohorts will be enrolled:

4.1.3.1 Cohort 2A – FLT3-ITD:

4.1.3.1.1 Patients with MDS, CMML or AML who are either:

4.1.3.1.1.1 Age 60 years or older and newly diagnosed, previously untreated. Prior therapy with hydroxyurea or single agent ara-C for the purpose of control of WBC is acceptable.

4.1.3.1.1.2 Age 18 years or older and with refractory or relapse disease who have received no more than

one prior treatment regimen and will be receiving first salvage. For this purposes, a second induction cycle with the same drugs used during the first cycle, consolidation chemotherapy or stem cell transplant in CR (or CRp or CRi) will be considered part of the prior regimen. Prior therapy for MDS (or other malignancies) is not considered a prior regimen for AML in patients who progress from MDS (or other malignancies).

4.1.3.1.2 Patients (any age) with MDS or CMML who received therapy with a hypomethylating agent and progress to AML are eligible regardless of any prior therapy for AML. The WHO classification will be used for AML.

4.1.3.1.3 Patients must have evidence of FLT3 ITD in their most recent assessment.

4.1.3.2 Cohort 2B – FLT3-WT:

4.1.3.2.1 Patients with MDS, CMML or AML who are either:

4.1.3.2.1.1 Age 60 years or older and newly diagnosed, previously untreated. Prior therapy with hydroxyurea or single agent ara-C for the purpose of control of WBC is acceptable.

4.1.3.2.1.2 Age 18 years or older and with refractory or relapse disease who have received no more than two prior treatment regimens and will be receiving second salvage, or who have received a prior SCT and will be receiving their first salvage. For this purposes, a second induction cycle with the same drugs used during the first cycle, consolidation chemotherapy or stem cell transplant in CR (or CRp or CRi) will be considered part of the prior regimen. Prior therapy for MDS (or other malignancies) is not considered a prior regimen for AML in patients who progress from MDS (or other malignancies).

4.1.3.2.2 Patients (any age) with MDS or CMML who received therapy with a hypomethylating agent and progress to AML are eligible at the time of diagnosis of AML regardless any prior therapy for AML. The WHO

classification will be used for AML.

4.1.3.2.3 Patients must have no evidence of FLT3 mutations in their most recent assessment.

4.1.4 For Phase I and II

4.1.4.1 ECOG Performance Status ≤2

4.1.4.2 Adequate liver (bilirubin ≤2x ULN, ALT ≤2.5x ULN) and renal (creatinine ≤2x ULN) function. For patients with suspected liver infiltration from leukemia, ALT should be </= 5 x ULN.

4.1.4.3 Serum potassium, magnesium, and calcium (normalized for albumin) levels should be at least within institutional normal limits.

4.1.4.4 Patients must provide written informed consent.

4.1.4.5 Patients must have been off chemotherapy for 2 weeks prior to entering this study, unless there is evidence of rapidly progressive disease, and must have recovered from the toxic effects of that therapy to at least grade 1. Use of hydroxyurea for patients with rapidly proliferative disease is allowed before the start of study therapy and for the first four weeks on therapy. The additional days of Hydrea after 28 is permitted as clinically indicated, on case by case basis and after discussion with the PI. Other agents given transiently with the intention to control rapid proliferation such as 1-2 doses of single agent ara-C or few doses of sorafenib are also allowed.

4.1.4.6 Women of childbearing potential must practice contraception. Women considered not of childbearing potential include any of the following: no menses for at least 2 years or menses within 2 years but amenorrheic for at least 2 months and luteinizing hormone (LH) and follicular stimulating hormone (FSH) values within normal range (according to definition of postmenopausal for laboratory used) or bilateral oophorectomy or radiation castration and amenorrheic for at least 3 months. Females of childbearing potential should practice effective methods of contraception Effective methods of contraception include barrier methods (e.g., condoms, diaphragm), spermicidal jelly or foam, oral, depo provera, or injectable contraceptives, intrauterine devices, tubal ligation, and abstinence. Male patients with female partners who are of childbearing potential should also practice contraception.

4.1.5 Negative urine or serum pregnancy test.

4.2 Exclusion Criteria

4.2.1 Patients with known allergy or hypersensitivity to quizartinib, mannitol, AZA, cytarabine or any of their components.

4.2.2 Patients with electrolyte abnormalities at study entry defined as follows:

4.2.2.1 Serum potassium < 3.5 mEq/L despite supplementation, or > 5.5 mEq/L.

4.2.2.2 Serum magnesium above or below the institutional normal limit despite adequate management.

4.2.2.3 Serum calcium (corrected for albumin levels) above or below institutional normal limit despite adequate management.

4.2.3 Patients with known significant impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of quizartinib.

4.2.4 Patients with any other known disease concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, cardiovascular disease including congestive heart failure, myocardial infarction within 6 months and poorly controlled hypertension, chronic renal disease, or active uncontrolled infection) which could compromise participation in the study. Patients with current active malignancies or any remission for < 6 months, except patients with carcinoma in situ or with non-melanoma skin cancer who may have active disease or be in remission for less than 6 months.

4.2.5 Patients with a known confirmed diagnosis of HIV infection or active viral hepatitis.

4.2.6 Patients who have had any major surgical procedure within 14 days of Day 1.

4.2.7 Patients with known malignant disease of the central nervous system.

4.2.8 Impaired cardiac function including any of the following

4.2.8.1 Screening ECG with a QTc >450 msec. The QTc interval will be calculated by Fridericia's correction factor (QTcF) at Screening and on Day 5 prior to the first dose of AC220. The QTcF will be derived from the average QTcF in triplicate. If QTcF >450 msec on Day 5, AC220 will not be given.

4.2.8.2 Patients with congenital long QT syndrome

4.2.8.3 History or presence of sustained ventricular tachycardia requiring medical intervention

4.2.8.4 Any history of clinically significant ventricular fibrillation or torsades de pointes

4.2.8.5 Known history of second or third degree heart block (may be eligible if the patient currently has a pacemaker)

4.2.8.6 Sustained heart rate of <50/minute on pre-entry ECG

4.2.8.7 Right bundle branch block + left anterior hemiblock (bifascicular block)

4.2.8.8 Patients with myocardial infarction or unstable angina within 6 months prior to starting study drug

4.2.8.9 CHF NY Heart Association class III or IV

4.2.8.10 Atrial fibrillation documented within 2 weeks prior to first dose of study drug.

4.2.8.11 Patients who require treatment with concomitant drugs that prolong QT/QTc interval or strong CYP3A4 inhibitors or inducers with the exception of antibiotics, antifungals, and antivirals that are used as standard of care to prevent or treat infections and other such drugs that are considered absolutely essential for the care of the subject.

4.2.9 Known family history of congenital long QT syndrome

5.0 TREATMENT PLAN

5.1 General

All patients will be registered through CORe. The objective will be to administer AZA or LDAC, and quizartinib at dose level 0 as defined in the Table below. We will first treat 6 patients at dose level -1. If no DLT is identified in >1 patient, this level is defined as MTD and up to 20 patients will be treated at this level with each treatment combination (AC220+ AZA or AC220 + LDAC) for the phase II portion. Other dose levels will be used for dose adjustments for toxicity during therapy.

Approximately 200 patients will be enrolled on this study.

5.2 Schedule

5.2.1 Patients will be treated according to the following schedule:

- AZA will be administered subcutaneously (SQ) or intravenously (IV) for 7 days of every cycle (Days 1-7) as determined by the treating physician. Both SQ and IV forms of administration are FDA approved and considered interchangeable. Patients may start receiving AZA IV and changed to SQ at any time (and vice versa) as needed based on patient and physician preference. AZA is administered in the clinic or chemotherapy administration area.

- Cytarabine will be administered SQ twice daily for 10 days of every cycle (Days 1-10) as determined by the treating physician. Cytarabine can be self-administered by the patient.

- Quizartinib will be administered orally daily for 28 days of every cycle. For the first cycle only, quizartinib will be administered starting on day 5 of the cycle. All subsequent cycles quizartinib will start concomitantly with AZA or cytarabine.

5.2.1.1 The starting and target dose is dose level 1.

Table 1(a): Dose reduction and escalation for Cytarabine + AC220 treatment arm

Dose Level	Cytarabine (mg BID x 10 days)	AC220 (mg/d, uninterrupted)
-3	10	30
-2	15	30
-1	20	30
1 (Starting and target dose)	20	60
2	20	90

Table 1(b): Dose reduction and escalation for AZA+ AC220 treatment arm

Dose Level	AZA ($mg/m^2/d$ x 7 days)	AC220 (mg/d, uninterrupted)
-3	25	30
-2	50	30
-1	75	30
1 (Starting and target dose)	75	60
2	75	90

5.2.1.2 Dose reductions beyond those mentioned in this table or different to the doses specified should be discussed with the PI and documentation of the justification recorded in the chart.

5.2.2 Patients will be assigned to treatment arm by physician's choice and according to the availability of slots.

5.2.2.1 Treatment arm is defined by the use of each of the possible chemotherapy agents (i.e., AZA + AC220 is one treatment arm and Cytarabine + AC220 is another treatment arm).

5.2.2.2 Patients in Cohort 2B (FLT3 WT) will only be treated with AZA + AC220. The combination with cytarabine will not be explored in this subset of patients in this study.

5.2.3 The first 6 patients on each cohort will receive dose level 1. At least 3 of the patients will be females.

5.2.3.1 A cohort is defined by each dose level.

5.2.4 Prior to advancing/changing cohorts a cohort summary will be completed and submitted to the Medical Monitor in the IND Office.

5.2.5 DLT is defined as any clinically significant non-hematologic adverse event or abnormal laboratory value occurring during the first cycle on study unless the event or laboratory value can clearly be determined to be unrelated to AC220 with the following exceptions:

- CTCAE Grade 3 or 4 AST (SGOT) or ALT (SGPT) that resolves to ≤ grade 2 in 14 days
- Grade 3 nausea, vomiting, or diarrhea that can be managed to ≤ grade 2 with standard antiemetic or antidiarrheal medications used at prescribed dose within 7 days of onset.
- Grade 4 vomiting, or diarrhea that can be managed to ≤ grade 3 with standard antiemetic or antidiarrheal medications used at prescribed dose within 48 hours of onset.
- Grade 3 or 4 electrolyte abnormalities will only be considered DLT if possibly related to study drug and not corrected by optimal replacement therapy within 48 hours.
- Grade 3 biochemical abnormalities of amylase or lipase without evidence of clinical pancreatitis.
- Grade 3 anorexia or fatigue.
- Grade 3 or 4 QTc prolongation will constitute DLT as follows: The QTc interval will be calculated by Fridericia's correction factor (QTcF). If the QTc is > 500 msec the EKG must be evaluated within 2 hours in triplicate and if ≥ grade 3 QTc prolongation is confirmed this will be considered a DLT.

Hematologic DLT is defined as grade ≥3 neutropenia and/or thrombocytopenia with a hypocellular bone marrow lasting for 6 weeks or more after the start of a course in the absence of residual leukemia (i.e., with less than 5% blasts). Anemia will not be considered for the definition of DLT.

5.2.5.1 If DLT occurs in ≥2/6 patients, this dose level would exceed the MTD, and an additional 6 patients will be treated at the next lower dose level. The dose level at which 0-1/6 patients experience DLT (but not higher than 60 mg/day) will be used to treat a maximum of 20 patients in each cohort in the phase II portion of the study. If ≥2/6 patients experience DLT at dose level -1 in any arm, dose level -2 may be explored for that arm(s).

5.2.5.2 If DLT is observed in 0 or 1/6 in dose level 1, this cohort will be expanded to treat up to 20 patients in the phase II portion of the study. If ≥ 2/6 patients experience DLT at dose level 1 for any arm of the study, the dose level at which </=1/6 patients have experienced DLT will be used for the phase II portion of the study for that arm.

5.2.5.3 If DLT is observed in 2/6 patients treated at dose level 1 in any arm, the study will continue at the quizartinib dose of 30 mg PO (dose level -1) for that arm to treat an additional 26 patients at this dose level in the phase II portion of the study.

5.2.5.4 Patients that are removed from study before day 28 for any reason other than toxicity and have not experienced DLT will be replaced.

5.2.5.5 DLT and MTD will be defined independently for each treatment arm (i.e., AC220 + AZA and AC220 + cytarabine).

5.2.6 One cycle of therapy is defined 7 days of AZA or 10 days of LDAC, and 28 days of quizartinib. Patients will receive one cycle of therapy every 4 weeks.

5.2.6.1 Cycles may be started early (but not earlier than day 21) for patients with active disease if judged in the best interest of the patient.

5.2.6.2 Subsequent cycles may be delayed for recovery of toxicity. Delays in start of subsequent cycles greater than 8 weeks should be discussed with the principal investigator.

5.2.6.3 Quizartinib therapy may continue if there is a delay in the start of the next cycle of AZA provided the delay is not due to toxicity possibly related to quizartinib.

5.2.6.4 Subsequent courses may be administered regardless of peripheral blood counts during the first 4 cycles and/or in the presence of residual leukemia. If prolonged myelosuppression (more than 60 days) with evidence of a hypocellular marrow (marrow cellularity less than 5% without evidence of leukemia) is observed subsequent courses of AZA may be given at the next lower dose. If the peripheral counts do not recover (ANC <1 $x10^9$/L and/or platelets <30 $x10^9$/L) but there is evidence of residual leukemia in the bone marrow, subsequent cycles can be administered at the discretion of the treating physician not earlier than 3 weeks after the prior cycle.

5.2.7 Quizartinib Administration

Quizartinib will be administered orally at a starting dose of 60 mg daily for the scheduled 28 days in each cycle.

5.2.7.1 If a dose is missed or vomited, the next dose should not be increased to account for missing a dose. The subject should take the next regular dose at the regularly scheduled time.

5.2.7.2 Treatment may be prolonged beyond the planned 28 days of each cycle if the start of the next cycle of AZA or LDAC is delayed. However, if there are adverse events that mandate treatment interruption or it is considered in the best interest of the patient for safety reasons to interrupt therapy, quizartinib administration can be transiently discontinued and re-started as per guidelines in section 6.0.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

1. Clinically significant progressive disease
2. Grade 4 QTc prolongation.
3. Intercurrent illness that prevents further administration of treatment

4. Patient request

5. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

6. Unacceptable toxicity that in the opinion of the investigator makes it unsafe to continue therapy. This includes patients with grade 4 non-hematological toxicity unless it reverses to grade ≤ 1 on or before 14 days of interrupting AC220.

5.3.1 It is planned that up to a total of 12 cycles of therapy will be administered for patients deriving benefit from this regimen. Continuation of therapy for patients completing 12 cycles of therapy may be considered on a case by case basis after discussion with the principal investigator.

5.3.2 A minimum of 1 full course (defined as the administration of AZA for 7 days or LDAC for 10 days, and quizartinib for 24 days for cycle one, i.e. days 5-28) will be required for a patient to be considered as having received an adequate trial to evaluate efficacy. All patients receiving at least one dose of any of the two drugs will be considered evaluable for toxicity.

5.4 Concomitant Medications

5.4.1 The use of any concomitant medication/therapies deemed necessary for patient supportive care and safety are permitted. Other anticancer agents including systemic chemotherapy, radiation therapy, or biologic response modifiers are not permitted during the study. No other investigational drug is allowed during the study. Patients must be off growth factors for five days in order to declare a neutrophil response. Antiemetics may be used for the prevention or treatment of nausea and vomiting.

5.4.2 The administration of drugs known to prolong QTc interval or strong CYP3A4 inhibitors should be administered with caution. Any drug known to inhibit or induce CYP3A4 will likely interact with quizartinib.

The list of drugs that can be associated with Torsades de Pointes or prolonged QT interval can be found online at:
http://www.azcert.org/medical-pros/drug-lists/bycategory.cfm.

The list of drugs that interact with CYP3A4 can be found online at:
http://medicine.iupui.edu/clinpharm/DDIs/ClinicalTable.aspx

5.4.3 Administration of other antineoplastic agents is prohibited for patients while on this study with the following exceptions: 1) Hydroxyurea may be

used for the first 28 days of therapy. The additional days of Hydrea after 28 days is permitted as clinically indicated, on case by case basis and after discussion with the PI . 2) Administration of intrathecal chemotherapy is allowed when clinically indicated.

6.0 DOSING DELAYS/DOSE MODIFICATIONS

6.1 Toxicity Directly Attributable to Study Drug

Patients experiencing unacceptable toxicity directly attributable to the study drugs should temporarily stop treatment according to the guidelines in the dose adjustment schema.

6.2 Toxicity Grading

Toxicity grading will be according to the NCI CTCAE, v4. To prevent unnecessary morbidity, the following guidelines for dose adjustment for drug-related toxicities are recommended.

6.3 Dose Reductions

Table 2(a): Dose reduction and escalation for Cytarabine + AC220 treatment arm

Dose Level	Cytarabine (mg BID x 10 days)	AC220 (mg/d, uninterrupted)
-3	10	30
-2	15	30
-1	20	30
1 (Starting and target dose)	20	60
2	20	90

Table 2(b): Dose reduction and escalation for AZA+ AC220 treatment arm

Dose Level	AZA (mg/m^2/d x 7 days)	AC220 (mg/d, uninterrupted)
-3	25	30
-2	50	30
-1	75	30
1 (Starting and target dose)	75	60
2	75	90

Dose reductions for non-hematologic toxicity possibly related to study drugs should be performed according to the following table:

Toxicity	Grade	Actions
Non-hematological (excludes myalgia/ arthralgia responding to treatment, inadequately treated nausea, vomiting and diarrhea, or electrolyte abnormalities unless not responding to optimal supplementation)	3	Hold therapy until recovery to Grade ≤ 1, then re-start and reduce to the next lower dose level. If toxicity recurs again, hold therapy until recovery to grade ≤1, then re-start and reduce one additional dose level. Dose reductions below dose level -3 will be considered on an individual basis after discussion with the principal investigator.
Non-hematological, study-drug related	4	Remove from study. An exception can be made if grade 4 toxicity reverses to Grade ≤ 1 on or before 14 days of interrupting study drug and re-dosing with AC220 is considered safe and in the best interest of the patient. In those instances treatment may be resumed with a 1 dose-level reduction after discussion with PI and Sponsor.
Non-hematological (excludes myalgia/ arthralgia responding to treatment, inadequately treated vomiting and diarrhea, or electrolyte abnormalities unless not responding to optimal supplementation)	Persistent 2 considered clinically significant or upon patient's request	Hold therapy until recovery to Grade ≤ 1, then re-start and reduce to the next lower dose level. If toxicity recurs again, hold therapy until recovery to grade ≤1, then re-start and reduce one additional dose level. Dose reductions below dose level -3 will be considered on an individual basis after discussion with the principal investigator.
Cardiac Toxicity	QTc > 450 and ≤ 480 msec	Check magnesium and potassium levels and correct any abnormalities. If possible, stop any medications that may prolong the QTc interval. Continue quizartinib at the same dose.

Cardiac Toxicity	QTc > 480 and ≤ 500 msec	Check magnesium and potassium levels and correct any abnormalities. If possible, stop any medications that may prolong the QTc interval. Decrease AC220 to next lowest dose level until QTc interval improves to ≤480 msec. AC220 dose may then be escalated to the previous dose if other external factors have been corrected and QTc has remained below 480 msec in at least 3 consecutive ECG separated for at least 2 weeks.
Cardiac Toxicity	QTc interval >500 msec	Check magnesium and potassium levels and correct any abnormalities. Interrupt AC220 and if possible any medications that may prolong the QTc interval. If QTc interval resolves to within 30 msec of baseline value within 7 days resume AC220 at the next lower dose level. If not resolved within 7 days, discontinue therapy permanently.
Cardiac Toxicity	QTc interval >500 or >60 ms change from baseline, ***and*** Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia (i.e., grade 4)	Discontinue therapy

6.3.1 Patients in whom the toxicity occurs or persists beyond the planned completion of drug administration for the cycle will have the dose reductions implemented in subsequent cycles provided the toxicity has resolved as specified in the table above.

6.3.2 If adverse events are clearly associated with one of the drugs, dose adjustments to only that agent may be performed.

6.3.3 Dose modifications different than those described above (e.g., dose reductions by 2 dose levels, decreased number of days of administration of either drug continuation of only quizartinib) may be implemented if judged in the best interest of the patient after discussion with the PI and documentation of the rationale for this action.

6.3.4 Dose adjustments for only of the drugs may be considered when the adverse event may be clearly associated with only one drug (e.g., QTc prolongation with quizartinib).

6.3.5 Patients in whom therapy is held for > 8 weeks due to toxicity will be removed from the trial.

6.4 Myelosuppression

Patients with leukemias and MDS usually present with abnormal peripheral blood counts at the time therapy is started and myelosuppression is an expected event during the course of therapy for acute leukemias and myelodysplastic syndromes. Thus, no dose adjustments or treatment interruptions for myelosuppression will be planned for the first 4 weeks of therapy. After this time, treatment interruptions and dose adjustments may be considered on an individual basis. The following guidelines can be used to consider treatment:

6.4.1 Patients with neutropenia or thrombocytopenia as a consequence of the disease do not require treatment interruptions for myelosuppression. Dose-reductions in these patients should be considered in an individual case and discussed with the PI. The following guidelines can be used for these patients:

6.4.1.1 Patients with a response and pre-cycle counts of neutrophils $>1x10^9$/L and platelets >50 $x10^9$/L who have sustained low counts of neutrophils <0.5 $x10^9$/L or a platelet count <20 x $x10^9$/L for more than 2 consecutive weeks in the current cycle, may receive a subsequent course at 1 dose level reduction. A reduction of 2 dose levels may be considered if the myelosuppression was deemed severe and life threatening by the treating physician, and if it is in the patient's best interest.

6.4.1.2 If there are persistent peripheral blood blasts, or the bone marrow shows >5% blasts, may continue treatment regardless of neutrophil and platelet count and give supportive care as needed.

6.4.1.3 If no marrow evidence of leukemia, may hold therapy until recovery of granulocytes to ≥ 1 $x10^9$/L and platelets ≥ 60 $x10^9$/L,

then resume at same or 1 lower dose level according to guidelines mentioned above.

6.5 Intrapatient Dose Escalation for Patients Treated at Dose Level -1 or Lower

Intrapatient dose escalation will be permitted for these patients provided:

6.5.1 Patient has completed ≥1 cycle at dose level -1 or lower, and

6.5.2 Patient has not experienced any grade 3 or higher non-hematologic toxicity, and

6.5.3 Patient has not experienced hematologic DLT, and

6.5.4 At least 3 patients have been treated at the next higher dose level and followed for at least 28 days without experiencing DLT.

6.5.5 Patients who have experienced no QTc prolongation above 480 msec in whom optimal anti-leukemia effect has not been reached may have the dose of quizartinib increased to by one dose level. Patients who have such dose escalation will have an EKG done 24 hours and 7 days after dose escalation. No dose escalation above 90 mg/day will be allowed.

6.5.6 Because of the increased risk of QTc prolongation in women treated at doses > 90 mg/day in prior studies with AC220, dose escalation above 90 mg/day is not allowed in women.

7.0 AGENT FORMULATION AND PROCUREMENT

7.1 Quizartinib (AC220)

7.1.1 Description

The chemical name of the investigational product, quizartinib, is N-(5-tert-Butyl-isoxazol-3-yl)-N′ -[4-[7-(2-morpholin-4-yl-ethoxy)imidazo[2,1-b][1,3]benzothiazol-2-yl]phenyl] urea dihydrochloride salt.

AC220 is supplied as tablets each containing 30 mg AC220 (26.5 mg free base) and 300 mg HPβCD. The tablets also contain microcrystalline cellulose, magnesium stearate, and Opadry® II 85F18422 white film coating. They are packaged in high density polyethylene bottles, each containing 30 tablets, with child-resistant caps.

7.1.2 Clinical Pharmacology

Analysis of human plasma samples demonstrate that AC220 is orally bioavailable with a long effective half-life, estimated to be about 1.5 days. In addition, AC220 exhibits minimal peak and trough variation of plasma levels with once daily dosing. In addition, a pharmacologically active metabolite, AC886, has been identified in plasma and urine samples, which has a slightly longer half-life of about 2 days. Since food has been shown to affect absorption in animals, AC220 should be taken in the morning on an empty stomach.

7.1.3 Potential Indications and Usage

AC220 is currently being evaluated as a treatment for patients with AML who are not candidates for standard therapy (Phase 1 study), or whose disease is refractory to, or has relapsed, following standard therapy in a multiple cohorts of patients (Phase 2 study) irrespective of age. Patients with and without FLT3 mutations are being investigated. It is planned to assess the safety and efficacy of AC220 as monotherapy and/or in combination with other agents for the treatment of AML and also in other oncology and disease indications.

7.1.4 Dosing

Each patient will receive or be instructed to take AC220 oral tablets at the assigned dose once a day on an empty stomach at least 1 hour before or 2 hours after a meal in the morning for 28 consecutive days as 1 cycle of treatment.

7.1.5 Selection and Timing of Dose

Each treatment cycle consists of 28 consecutive days. AC220 will be taken orally once a day on an empty stomach (i.e., 1 hour before or 2 hours after a meal) in the morning. The starting dose in the Phase 2 monotherapy study was 200 mg/day. Due to a higher than anticipated rate of QT prolongation, lower doses of 90mg/day in females and 135mg/day in males was pursued after the initial 17 subjects were enrolled out of a total of 333 subjects in the Phase 2 study.

7.1.6 Packaging and Labeling

Please refer to the Pharmacy Manual for packaging and labeling information.

7.1.7 Study Drug Preparation

Please refer to the Pharmacy Manual for packaging and labeling information.

7.2 **AZA and cytarabine:** these drugs are commercially available and will be handled as per the packet insert and standards in the MDACC investigational pharmacy.

7.3 **Disposition of unused drug:** all unused drug will be disposed of per institutional guidelines and procedures.

8.0 CORRELATIVE/SPECIAL STUDIES (Optional)

In patients treated with FLT3 inhibitors, the degree of FLT3 inhibition in vivo has been shown to be an important parameter in determining clinical response (Levis et al 2011 Blood 117:3294). In addition, the plasma levels of FLT3 ligand (FL) may influence the degree of in vivio inhibition (Sato et al 2011 Blood 117:3286). In patients treated with quizartinib and 5-azacitidine or low-dose AraC (LDAC), it is hypothesized that in vivo FLT3 inhibition, which may be impacted by FL levels, will influence clinical responses. Therefore, we propose to measure in vivo FLT3 inhibition and FL levels in patients enrolled on this trial. We will collect whole blood into a 10 cc heparinized (green top) vacutainer tube pre-treatment and on Days 1, 8, and 15 of each cycle up to the first 4 cycles (all ±2 days) ; (therefore 12 samples maximum per patient). For each time point, therefore, approximately 8 cc whole blood will be collected. The blood will be centrifuged to separate the cellular fraction, and the plasma (3-5 cc per sample) will be transferred into a separate tube for frozen storage. Samples will be shipped in batches every month to the laboratory of Dr. Mark Levis:

Mark Levis MD PhD
Cancer Research Building 1, Room 230
1650 Orleans Street
Baltimore, MD 21231

For analysis, frozen plasma will be thawed rapidly, then stored on ice. For FL levels, 200 microliters will be used in an ELISA-based assay system (R&D). For determination of in vivo FLT3 inhibition, the remaining plasma will be assayed using a Plasma Inhibitory Activity assay for FLT3 by incubating 1 cc plasma with the FLT3/ITD-expressing Molm14 cell line, then performing immunoblotting for phospho-FLT3 as described (Levis et al 2006 Blood 108:3477). The degree of in vivo FLT3 inhibition will be calculated as a percent of the baseline (from the baseline sample collected pre-treatment). Results will be entered into a spreadsheet and returned to the study PI when all samples have been analyzed.

9.0 PATIENT EVALUATION (See Appendix AA)

9.1 Pre-Treatment Evaluation

All pretreatment studies should be obtained within 14 days of entry into the trial, unless otherwise stated.

9.1.1 A complete history and physical, documentation of all measurable disease, concomitant medications and performance status.

9.1.2 CBC, platelet count, differential (differential can be omitted if WBC is ≤0.5 $x10^9$/L).

9.1.3 Creatinine, total bilirubin, ALT, potassium, magnesium, calcium, sodium, chloride, CO_2, uric acid, phosphorus, and BUN.

9.1.4 Pregnancy test (urine or plasma) in females of childbearing potential, should be performed 48 hours before initiation of study.

9.1.5 Bone marrow aspirate during the last 21 days preceding study initiation. Cytogenetics will be obtained prior to therapy (results from prior analysis can be used for this purpose).

9.1.6 Evaluation of FLT3 if not done within 90 days, provided there has been no intervening therapy other than hydroxyurea or isolated single-agent doses of cytarabine.

9.1.7 EKG and ECHO and/or MUGA will be performed before initiating study treatment if not done within 28 days.

9.1.8 Pretreatment optional correlative studies (see Section 8.0)

9.2 Evaluation During Treatment

9.2.1 Physical exam at the start of each cycle (± 5 days) for the first 2 cycles, then every 2-3 cycles and documentation of all concomitant medications.

9.2.1.1 Patients will keep a diary for drug administration of self-administered drugs (cytarabine and AC220)

9.2.2 CBC, platelet count, differential once weekly for the first 3 cycles, then every 2-4 weeks (all ±2 days) (differential can be omitted if WBC is ≤0.5 $x10^9$/L)

9.2.3 Creatinine, total bilirubin, ALT, K, Mg, Ca, sodium, chloride, and CO2 on day 5 prior to start of AC220, then weekly for the first 3 cycles, then every 2-4 weeks(all ±2 days) .

9.2.4 Bone marrow aspiration on day 28 (+/- 7 days) of cycle 1, then every 1-3

cycles.

9.2.5 EKG will be performed on day 1, day 5, day 8, and day 12 of the first cycle (all ±2 days) Day 1 and day 8 ECG are collected pre-dose; day 5 are collected pre-dose and 4 hrs (+/- 2 hrs) post-dose; day 12 are collected 4 hrs (+/- 2 hrs) post-dose. Then ECG to be done on day 1 of each cycle for cycles 2 and 3 before initiation of quizartinib and 4 hr (+/-2 hrs) post dose, then pre-dose every 2-3 cycles. EKG may be omitted if patient has not received study drug (AC220) in the 48 hrs preceeding the time when EKG is done. EKG for the first 3 cycles will be done in triplicate. Day 5 pre AC-220 will be used as baseline.

9.2.5.1 On patients in whom quizartinib is escalated to 90 mg daily as described in section 6.5.5 or who start therapy with a drug that may prolong QT/QTc or that is a strong CYP3A4 inhibitor or inducer , an EKG will be required 24 hrs (±6 hrs), 7 days (±2 days) and 14 days (±2 days) after the dose increase or after the start of such drug.

9.2.5.2 Patients who had their dose reduced because of QTc prolongation and have their dose re-escalated as described in section 6.3 will have an EKG repeated 24 hrs (±6 hrs) and 7 days (± 2 days) after re-escalation.

9.2.5.3 The QTc interval will be calculated by Fridericia's correction factor (QTcF). The 12–lead EKG evaluation will consist of 3 separate EKGs obtained at least 5 minutes apart.

9.2.6 Correlative studies on days 1, 8 and 15 of each cycle up to the first 4 cycles (all ±2 days). (Optional – See Section 8.0).

9.2.7 For patients that remain on study with no significant toxicity for more than 6 months, subsequent evaluations during study may be modified after discussion with the principal investigator. These include a decrease in frequency of standard laboratory tests to once every cycle, bone marrow aspirations to every 6-12 months (or as clinically indicated).

Outside Physician Participation During Treatment

1. MDACC Physician communication with the outside physician is required prior to the patient returning to the local physician.
   *This will be documented in the patient record
2. A letter to the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care (Appendix E)

3. Protocol required evaluations outside MDACC will be documented by telephone, fax or e-mail. Fax and/or e-mail will be dated and signed by the MDACC physician, indicating that they have reviewed it.
4. Changes in drug dose and/or schedule must be discussed with and approved by the MDACC physician investigator, or their representative prior to initiation, and will be documented in the patient record.
5. A copy of the informed consent, protocol abstract, treatment schema and evaluation during treatment will be provided to the local physician.
6. Documentation to be provided by the local physician will include drug administration records, progress notes, reports of protocol required laboratory and diagnostic studies and documentation of any hospitalizations.
7. The home physician will be requested to report to the MDACC physician investigator all life threatening events within 24 hours of documented occurrence.
8. Patients will return to MDACC approximately every month.

10.0 CRITERIA FOR RESPONSE

Criteria for response will be as per the international working group for MDS and AML.

10.1 Response Criteria for MDS

Response criteria will be according to the International Working Group (Blood 2006; 108: 419-425). Responders are patients who obtain a CR, CRi, or PR, with or without cytogenetic response, hematologic improvements, and morphologic leukemia-free state. Briefly, criteria are as follows:

10.1.1 *Morphologic Complete Response (CR)*

- **Peripheral blood counts:**
  No circulating blasts
  Neutrophil count $\geq 1.0 \times 10^9/L$
  Platelet count $\geq 100 \times 10^9/L$

- **Bone marrow aspirate and biopsy:**
  $\leq$ 5% blasts
  No extramedullary leukemia

10.1.2 Partial Response (PR)

- All CR criteria if abnormal before treatment except:
- ≥50 % reduction in bone marrow blast but still >5%

10.1.3 Marrow CR

- Bone marrow: ≤5% myeloblasts and decrease by ≥50% over pretreatment

- Peripheral blood: if HI responses, they will be noted in addition to marrow CR

10.1.4 Hematologic Improvement (HI): Hematologic response must be described by the number of positively affected cell lines.

- **Erythroid response (E)** (pretreatment Hgb <11 g/dL)
  Hgb increase by ≥1.5 g/dL

- **Platelet response (P)** (pretreatment platelets <100 x10^9/L)
  Absolute increase of ≥30 x 10^9/L for patients starting with > 20 x 10^9/L platelets
  Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%

- **Neutrophil response (N)** (pretreatment ANC <1.0 x10^9/L)
  At least 100% increase and an absolute increase > 0.5 x 10^9/L

10.2 Response Criteria for AML

Response criteria will be modified from the International Working Group for AML (JCO 2003; 21: 4642-9). Responders are patients who obtain a CR, CRi, or PR, with or without cytogenetic response, hematologic improvements, and morphologic leukemia-free state. Briefly, criteria are as follows:

10.2.1 ***Complete remission (CR):***

- **Peripheral blood counts:**
  No circulating blasts
  Neutrophil count ≥1.0 x10^9/L
  Platelet count ≥100 x10^9/L

- **Bone marrow aspirate and biopsy:**
  ≤5% blasts
  No Auer rods
  No extramedullary leukemia

10.2.2 Complete remission with incomplete blood count recovery (CRi):

- **Peripheral blood counts:**
  No circulating blasts
  Neutrophil count <1.0 x10^9/L, and/or
  Platelet count <100 x10^9/L

- **Bone marrow aspirate and biopsy:**
  ≤ 5% blasts

No Auer rods
No extramedullary leukemia

10.2.3 Partial remission:

- All CR criteria if abnormal before treatment except:
- ≥50 % reduction in bone marrow blast but still >5%

10.2.4 Morphologic leukemia-free state:

- Bone marrow: ≤≤5% myeloblasts

10.2.5 Hematologic Improvement (HI): Hematologic response must be described by the number of positively affected cell lines.

- **Erythroid response (E)** (pretreatment Hgb <11 g/dL)
  Hgb increase by ≥1.5 g/dL

- **Platelet response (P)** (pretreatment platelets <100 x10^9/L)
  Absolute increase of ≥30 x 10^9/L for patients starting with > 20 x 10^9/L platelets
  Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%

- **Neutrophil response (N)** (pretreatment ANC <1.0 x10^9/L)
  At least 100% increase and an absolute increase > 0.5 x 10^9/L

- **Blast response (Bl)** a ≥50% reduction in blast percentage in bone marrow and/or ≥50% reduction in peripheral blood total blast count.

11.0 ADVERSE EVENT REPORTING

11.1 Adverse event is any untoward medical occurrence that may present during treatment with a pharmaceutical product but which does not necessarily have a causal relationship with this treatment. Adverse drug reaction is a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function.

Assessing causal connections between agents and disease is fundamental to the understanding of adverse drug reactions. In general, a drug may be considered a contributory cause of an adverse event if, had the drug not been administered, 1) the event would not have happened at all, 2) the event would have occurred later than it actually did, or 3) the event would have been less severe.

The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

11.2 PDMS/CORe will be used as the electronic case report form for this protocol. Adverse events will be documented in the medical record and entered into PDMS/CORe.

11.3 Adverse Events (AEs) will be evaluated according to the latest CTC version and documented in medical record. All suspected adverse drug reactions (SADR) will be recorded in the Case Report Form (CRF). Expected events during leukemia therapy are:

11.3.1 Myelosuppression related events (due to disease or leukemia therapy)

11.3.1.1 febrile or infection episodes not requiring management in the intensive care unit

11.3.1.2 epistaxis or bleeding except for catastrophic CNS or pulmonary hemorrhage

11.3.1.3 anemia, neutropenia, lymphopenia, thrombocytopenia, leukopenia

11.3.2 Disease related events

11.3.2.1 symptoms associated with anemia

11.3.2.1.1 fatigue

11.3.2.1.2 weakness

11.3.2.1.3 shortness of breath

11.3.2.2 electrolyte abnormalities (sodium, potassium, bicarbonate, CO2, magnesium)

11.3.2.3 chemistry abnormalities (LDH, phosphorus, calcium, BUN, protein, albumin, uric acid, alkaline phosphatase, glucose)

11.3.2.4 coagulation abnormalities

11.3.2.5 disease specific therapy (induction, maintenance, salvage, or stem cell therapy)

11.3.2.6 alopecia

11.3.2.7 bone, joint, or muscle pain

11.3.2.8 liver function test abnormalities associated with infection or disease progression

11.3.2.9 disease progression

11.3.2.10 abnormal hematologic values

11.3.3 General therapy related events

11.3.3.1 catheter related events

11.3.3.2 renal failure related to tumor lysis syndrome or antibiotic/ antifungal therapy

11.3.3.3 rash related to antibiotic use

11.3.4 Hospitalization for the management of any of the above expected events

11.4 Abnormal hematologic values will not be recorded on the case report form. For abnormal chemical values, the apogee or nadir (whichever is appropriate) will be reported per course on the case report form.

11.5 Serious Adverse Event Reporting (SAE)

SAE reports should be addressed to DSI Clinical Safety and Pharmacovigilance (CSPV) via email (CSPV-Clinical@dsi.com) or facsimile (732-906-9621). The PI will use Institution's standard forms for reporting SAEs (Serious Adverse Event Reporting (SAVER) form) and pregnancies (Exposure In Utero (EIU) form) in accordance with ICH guidance. DSI's awareness date is the date a SAE is received from the PI. The PI will provide DSI CSPV with final CIOMS report by email (CSPVClinical@dsi.com) or facsimile (732-906-9621) in accordance with the following timeframe: (a) Fatal or life-threatening and related SAEs shall be reported within seven (7) calendar days of the date of awareness (b) All other SAEs shall be reported within fifteen (15) calendar days of the date of awareness. SAE submissions to Celgene within 24 hours of becoming aware of the event will be reported by fax to Celgene Drug Safety (908-673-9115). The reporting period ends thirty (30) days after discontinuation of drug, or Completion of subject's participation. In addition, PI must notify Celgene of any SAE that may occur after this time period which the PI believes to be certainly, probably or possibly related to Study Drug. Information not available at the time of the initial report (e.g., an

end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required.

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- **Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the INDIND Sponsor, IND Office.**

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug and those that constitute a clinically important increase in the rate of serious adverse reactions over those listed in the protocol or Investigator Brochure, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office. -

- **Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB. Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and**

laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

- **Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.**

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

MDACC Reporting Requirements

All SAEs will be reported to the IRB according to MDACC guidelines. Serious Adverse Events will be submitted electronically via the eSAE system to the IND Office and will be submitted to the FDA by the Safety Coordinator according to 21 CFR 312.32.

Investigator Communication with Supporting Companies:

Any serious suspected unexpected adverse reaction (where SAE is not reported in the Investigator Brochure and is considered to be related to study drug) will be reported to DSI Clinical Safety and Pharmacovigilance (CSPV) via email (CSPV-Clinical@dsi.com) or facsimile (732-906-9621) and by fax to Celgene Drug Safety (908-673-9115) only if related to Vidaza. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required.

12.0 STATISTICAL CONSIDERATIONS[48]

12.1 Overview

This is a phase I/II study of two combination therapies in patients with AML or MDS: AC220 + 5-azacytidine, and AC220 + low-dose cytarabine. Patients will be assigned to the two treatments based on physicians' choice. The two treatments will be evaluated separately.

12.2 Phase I

The primary objective of the phase I portion of the study is to determine the MTD of each combination therapy. The dose schedule is listed in table 1 of section 5.2.1. Up to 2 dose levels of AC220 will be tested in each combination with a fixed dose of the other drug. Initially, 6 patients will be treated at dose level1, if ≤1 patients experience DLT at dose level1, then 6 subsequent patients will be treated at the next higher dose level, dose level2. If ≤1 patients experience DLTs in dose level2, this level will be defined as the MTD and will be used in the phase II portion of the study. If >1 patients experience DLTs at dose level1, then the 6 subsequent patients will be treated at the next lower dose level, level -1. The MTD is defined as the highest dose at which no more than 1 patient experiences DLTs. For each combination therapy, 12 patients will be evaluated. The total sample size for the phase I portion will be 24.

12.3 Phase II

A: Cohort 2A – FLT3-ITD

The primary endpoint of the phase II portion of the study is overall response (CR+Cri+PR+HI) (first 2 treatment courses). It would be encouraging if the overall response rate reaches 50%.

Treatment with AC220 + low-dose cytarabine (Cohort 2A-FLT3-ITD)

Patients enrolled in the treatment of AC220 + low-dose cytarabine could be newly diagnosed or previously treated. A total of 26 patients (including 6 patients treated at MTD in Phase I) will be treated with this treatment combination. The toxicity and efficacy (CR+Cri+PR+HI) 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 and futility based on the following stopping rules. If the trial for this treatment continues until 26 patients are evaluated, and assuming the overall response rate is 50%, then the 95% credible interval for overall response rate would be (0.32, 0.68).

12.3.1 Overall response

Response will be monitored closely in patients using the method of Thall et al (1995). Denote the probability of overall response by P_E. We assume $P_E \sim$ beta (1, 1). We will stop the trial of this treatment if at any point Pr ($P_E < 0.5 \mid$ data) > 0.98. That is, we will stop the treatment of this combination if, at any time during the study, we determine that there is more than 98% chance that the response rate is less than 50%. The trial will be stopped if (the number of response observed / among number of patients) ≤ 1/10, 2/12, 3/15, 4/18, 5/21, and 6/24.

12.3.2 Toxicity

Toxicity will be monitored closely using the method of Thall et al (1995). Denote the probability of toxicity by θ_E, where toxicity is defined as DLTs. We assume $\theta_E \sim$ beta (0.6, 1.4). We will stop the trail if at any point $Pr(\theta_E > 0.30 \mid \text{data}) > 0.9$. That is, we will stop the trial of this combination therapy if, at any time during the study, we determine that there is more than 90% chance that the toxicity rate is more than 30%. The trial will be stopped if (the number of toxicity observed / among number of patients) ≥ 6/11, 7/14, 8/16, 9/19, 10/22 and 11/24.

Table 1 presents operating characteristics for the study design.

Table 1: Operating characteristics

Response rate	Toxicity rate	Probability of early stopping	Average number of patients
0.2	0.1	0.862	15.01
	0.2	0.864	14.94
	0.3	0.884	14.48
	0.4	0.929	13.37
	0.5	0.974	11.95
0.3	0.1	0.497	20.31
	0.2	0.508	20.14
	0.3	0.579	19.11
	0.4	0.743	16.64
	0.5	0.907	13.62
0.4	0.1	0.175	24.02
	0.2	0.192	23.79
	0.3	0.309	23.33
	0.4	0.577	18.88
	0.5	0.847	14.72
0.5	0.1	0.038	25.54
	0.2	0.058	25.29
	0.3	0.194	23.65
	0.4	0.507	19.79
	0.5	0.822	15.16
0.6	0.1	0.005	25.93
	0.2	0.026	25.67
	0.3	0.167	23.99
	0.4	0.49	20.02
	0.5	0.816	15.27

MultcLean V1.2 developed in the Department of Biostatistics MDACC was used to calculate the stopping boundaries for futility and toxicity monitoring rules.

Treatment with AC220 + 5-azacytidine (Cohort 2A-FLT3-ITD)

Up to 50 newly diagnosed patients (including patients treated at the same dose in phase I) and up to 50 previously treated patients (including patients treated at the same dose in phase I) will be treated with AC220 + 5-azacytidine. The analysis for the two groups of patients will be completed separately.

The study will monitor futility and safety for each patient group and the monitoring will begin when 15 patients have been enrolled in that group, and thereafter by a cohort of 5 patients. The trial in one group will be stopped early if at any time point the data in that group suggest
$Pr(P_E < 0.5 \mid data) > 0.98$ or
$Pr(\theta_E > 0.30 \mid data) > 0.9$

Where P_E denote the response rate for experimental treatment, and θ_E denotes the toxicity rate for the experimental treatment combination. Assume $P_E \sim$ prior beta (1,1) and $\theta_E \sim$ prior beta(0.6, 1.4).

That is, the trial in one group will be stopped early for futility if the data for that group indicate that there is more than 98% probability that the response rate is less than 50% or the trial for one group will be stopped early for toxicity if the data that group indicate that there is more than 90% probability that the toxicity rate (i.e. DLT rate) is more than 30%.

The corresponding stopping boundaries for futility is: the trial in one group will be stopped early if (the number of response observed / among number of patients) in that group ≤ 3/15, 5/20, 7/25, 9/30, 11/35, 13/40 and 15/45.

The corresponding stopping boundaries for toxicity is: The trial will be stopped early in one group if (the number of patients experienced DLTs / among number of patients) in that group ≥ 8/15, 9/20, 11/25, 13/30, 15/35, 16/40 and 18/45.

Table 2 presents the operating characteristics for the above design.

Table 2: OCs for the design for cohort 2A with AC220 + 5-AZA

Response rate	Toxicity rate	Probability of early stopping	Average number of patients
0.2	0.1	0.993	18.6
	0.2	0.993	18.6

	0.3	0.994	18.2
	0.4	0.998	17.4
	0.5	1	16.2
0.35	0.1	0.584	35.8
	0.2	0.59	35.5
	0.3	0.669	32.9
	0.4	0.865	26
	0.5	0.983	19.4
0.5	0.1	0.055	48.6
	0.2	0.069	48.2
	0.3	0.247	43.7
	0.4	0.694	31.7
	0.5	0.96	21
0.65	0.1	0.009	50
	0.2	0.016	49.6
	0.3	0.204	44.8
	0.4	0.676	32.4
	0.5	0.958	21.2

If the trial in one group continues until 50 patients are evaluated, and assuming the overall response rate is 50%, then the 95% credible interval for overall response rate would be (0.36, 0.64).

B: Cohort 2B – FLT3-WT

Up to 50 patients FLT3-WT will be treated AC220 + 5-azacytidine. The primary objective is to determine the over response. In a recent phase 2 study, we observed a single agent AC220 response of 30% in a salvage setting. We would like to have a 15% improvement (i.e. response rate of 45%) under the combination treatment in this study.

The study will also monitor futility and safety for this cohort. Monitoring will begin when 15 patients have been enrolled in that group, and thereafter by a cohort of 5 patients. The trial in this cohort will be stopped early if at any time point the data in that group suggest

$Pr(P_E < 0.15 + P_S \mid data) > 0.98$ or
$Pr(\theta_E > 0.30 \mid data) > 0.9$

Where P_E and P_S denote the response rate for experimental and standard treatment, respectively, and θ_E denotes the toxicity rate for the experimental treatment combination. Assume $P_E \sim$ prior beta(0.6, 1.4) and $\theta_E \sim$ prior beta(0.6, 1.4).

That is, the trial in this cohort will be stopped early for futility if the data indicate that there is more than 98% probability that the response rate is not improved by 15% or the trial will be stopped early for toxicity if the data indicate that there is more than 90% probability that the toxicity rate (i.e. DLT rate) is more than 30%. The corresponding stopping boundaries for futility is: the trial in this cohort will be stopped early if (the number of response observed / among number of patients) in this cohort $\leq$ 3/15, 4/20, 6/25, 8/30, 10/35, 11/40 and 13/45.

The corresponding stopping boundaries for toxicity is: The trial will be stopped early in this cohort if (the number of patients experienced DLTs / among number of patients) in this cohort $\geq$ 8/15, 9/20, 11/25, 13/30, 15/35, 16/40 and 18/45.

Table 3 presents the operating characteristics for the above design.

Table 3: OCs for the design for cohort 2B

Response rate	Toxicity rate	Probability of early stopping	Average number of patients
0.3	0.1	0.64	33
0.3	0.3	0.72	30.6
0.3	0.5	0.99	18.8
0.45	0.1	0.08	47.8
0.45	0.3	0.27	43
0.45	0.5	0.96	20.9
0.6	0.1	0.002	49.9
0.6	0.3	0.21	44.8
0.6	0.5	0.96	21.2

If the trial in one group continues until 50 patients are evaluated, and assuming the overall response rate is 45%, then the 95% credible interval for overall response rate would be (0.31, 0.58).

12.4 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 and its 95% credible intervals will be estimated.

13.0 PROTOCOL ADMINISTRATION

Protocol amendments

Changes to the protocol will be made only when protocol amendments have been signed by the principal investigator and approved by the sponsor and the IRB of the study center.

Archival of data

All patient data (including source data) generated in connection with this study will be kept in the archives of the MDACC for at least 15 years after the study has been completed. All data will be available for inspection by company representatives of the Medical Department and by regulatory authorities.

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Appendix AA – Schedule of Events*

Assessment	**Pre-Treatment Evaluation** (procedures to be performed within 14 days prior to study drug administration except where indicated)	**Cycle 1 and 2 - Day 1 (+/- 5 days), then every 2-3 cycles until end of Month 6**	**Once weekly during Cycles 1-3, then every 2-4 weeks until end of Month 6 (all ±2 days)**	**Cycle 1 Day 28 (+/- 7 days), then every 1-3 cycles until end of Month 6**	**Cycle 1-4, Days 1, 8, and 15 (all ±2 days)**
Complete History[a]	X				
Physical Examination	X	X			
Concomitant Medications	X	X			
Performance Status	X				
CBC, Platelet Count, Differential[b]	X		X		
Serum Chemistry	X[C]		X[j]		
Urine or Serum Pregnancy Test (WCBP)[d]	X				
Bone Marrow Aspiration	X[e]			X	
FLT3 Evaluation[f]	X				
EKG, ECHO, and/or MUGA[g]	X				
Correlative Studies[h]	X				X
Drug Administration Diary		X[i]			
EKG		X[k]			

* For patients that remain on study with no significant toxicity for more than 6 months, subsequent evaluations during study may be modified after discussion with the principal investigator. These include a decrease in frequency of standard laboratory tests to once every cycle, bone marrow aspirations to every 6-12 months (or as clinically indicated).

[a] documentation of all measureable disease

[b] differential can be omitted if WBC is $\leq 0.5 \times 10^9$/L

[c] creatinine, total bilirubin, ALT, potassium, magnesium, calcium, sodium, chloride, CO_2, uric acid, phosphorus, BUN

[d] 48 hours before initiation of study

[e] during the last ~~14~~ 21 days preceding study initiation. Cytogenetics will be obtained prior to therapy (results from prior analysis can be used for this purpose)

[f] if not done within 90 days, provided there has been no intervening therapy other than hydroxyurea or isolated single-agent doses of cytarabine.

[g] before initiating study treatment if not done within 28 days

[h] see Protocol Section 8.0

[i] drug administration of self-administered drugs (cytarabine and AC220)

[j] creatinine, total bilirubin, ALT, K, Mg, Ca, sodium, chloride, and CO2; on day 5 prior to start of AC220 and on day 6 as clinically indicated

[k] EKG will be performed on day 1, day 5, day 8, and day 12 of the first cycle (all ±2 days) Day 1 and day 8 ECG are collected pre-dose; day 5 are collected pre-dose and 4 hrs (+/- 2 hrs) post-dose; day 12 are collected 4 hrs (+/- 2 hrs) post-dose. Then ECG to be done on day 1 of each cycle for cycles 2 and 3 before initiation of quizartinib and 4 hr (+/-2 hrs) post dose, then pre-dose every 2-3 cycles. EKG may be omitted if patient has not received study drug (AC220) in the 48 hrs preceeding the time when EKG is done. EKG for the first 3 cycles will be done in triplicate. Day 5 pre AC-220 will be used as baseline.