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Protocol Page

Phase I-II Study of Low-Dose Azacitidine (Vidaza) in Patients With Chronic Myeloid Leukemia who Have Minimal Residual Disease While Receiving Therapy with Tyrosine Kinase Inhibitors (VZ-CML-PI-0236)
2011-0254

Core Protocol Information

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Which Committee will review this protocol?

(•) The Clinical Research Committee - (CRC)

Protocol Body

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Study Product:	Azacitidine
Protocol Number:	2011-0254
Coordinating Center:	MD Anderson Cancer Center 1515 Holcombe Blvd. Houston, TX 77030

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Phase I-II Study of Low-Dose Azacitidine (Vidaza) in Patients With Chronic Myeloid Leukemia who Have Minimal Residual Disease While Receiving Therapy with Tyrosine Kinase Inhibitors (VZ-CML-PI-0236)

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Study Product: *Azacitidine*

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Coordinating Center: *MD Anderson Cancer Center*
1515 Holcombe Blvd., Unit 428
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1. OBJECTIVES

1.1. Primary Objective:

1.1.1. **Phase I**: To determine the DLT and MTD of the combination of azacitidine (AZA) and a tyrosine kinase inhibitor (TKI) in patients with chronic myeloid leukemia (CML).

1.1.2. **Phase II**: To determine the clinical activity of the combination of AZA and a TKI in patients with CML in complete cytogenetic remission (CCyR) with minimal residual disease.

1.2. Secondary Objectives:

1.2.1. **Phase I**

1.2.1.1. To determine the clinical activity of the combination of AZA and a TKI in patients with CML

1.2.2. **Phase II**

1.2.2.1. To determine the safety of the combination of AZA and a TKI in patients with CML in CCyR with minimal residual disease.

1.2.3. **Both phases**

1.2.3.1. Determine the effect of therapy over DNA methylation

1.2.3.2. Investigate the correlation of DNA methylation and response to therapy

2. BACKGROUND

2.1. Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a pluripotent stem cell disorder characterized by the presence of the Philadelphia (Ph) chromosome in the leukemic cells. The Ph chromosome results from a (9;22) translocation in which the c-*ABL* oncogene has moved from chromosome 9 into the *BCR* (breakpoint cluster region) gene on chromosome 22, resulting in a chimeric *BCR-ABL* gene [1-3]. This is the causative abnormality in CML. The fused gene encodes an 8.5 kb chimeric mRNA [4,5], which is translated into a 210-kDa protein [6]. This p210 BCR-ABL protein functions as a constitutively activated tyrosine kinase and is uniquely present in the leukemic cells of CML patients [7]. The breakpoint in the *BCR* gene occurs either between *BCR* exon b2 and b3 or between *BCR* exons b3 and b4. Therefore, in the mature *BCR-ABL* mRNA, either b2 or b3 is spliced to *ABL* exon a2, which results in two alternative chimeric p210 BCR-ABL proteins, with either a B2A2 or B3A2 junction [8].

2.2. Tyrosine Kinase Inhibitor (TKI) Therapy

Imatinib is a low molecular weight phenylaminopyrimidine designed to selectively inhibit BCR-ABL tyrosine kinase activity [9], and is now the standard therapy for newly diagnosed patients with CML who do not undergo allogeneic stem cell transplant [10].

Results of the pivotal IRIS trial have shown that over 80% of patients achieve a CCyR, with most of these responses being durable. After 7 years of follow-up, the event-free survival rate is 81% and the survival free from transformation to accelerated or blast phase is 92%.[11] For patients who develop resistance or intolerance to imatinib, effective therapy with second generation tyrosine kinase inhibitors has been developed. Two of these agents (dasatinib and niloitinib) are currently available, and others are under development (e.g, bosutinib). Approximately 50% of patients treated with these agents after imatinib failure achieve a CCyR, and these responses are also durable in most patients.

Several reports have shown that, among patients who achieve a CCyR with imatinib or other TKI, those who achieve a MMR (ie, at 18 months from the start of therapy) have an improved EFS and survival free from transformation to accelerated or blast phase compared to those with CCyR but no MMR (7-year EFS 95% vs 86%, respectively).[12] In this same analysis, none of the patients who had achieved a MMR transformed to accelerated or blast phase. Recently, it has been suggested that achieving a complete molecular response (CMR) further improves the long-term outcome of patents after treatment with imatinib. In one series, patients who had an MMR had a shorter relapse-free survival (median 44 months) compared to those that achieved CMR (median not reached, with a hazard ratio for relapse 11%.[13] Similar results were reported from our institution, where patients with sustained complete molecular response have an estimate EFS at 7-years of 95%, compared to 85% for those with sustained MMR but no CMR, and 50% for those with CCyR but no MMR.[14] Thus, improving the molecular response of patients who have already achieved a CCyR with TKI has become an important goal of therapy. It has been suggested that patients who achieve CMR may discontinue therapy with imatinib, an important goal for patients. However, discontinuation of imatinib among patient who achieve CMR results in relapse in over 50% of patients.[15] Thus additional measures are needed to make treatment discontinuation a safer proposition.

2.3. **Methylation and Hypomethylating Agents in CML**

Increased methylation has been documented in CML and it plays a role in disease progression. The Pa promoter of Abl and several other key genes are hypermethylated in a significant percentage of patients. Methylation of this promoter increases with disease progression. Some studies have suggested an association of hypermethylation with worse prognosis, although this is still controversial.[16-19] The p15 gene is also hypermethylated as CML progresses, with differential patterns of hypermethylation for myeloid and lymphoid blastic phases.[16] Hypermethylation of Caderin-13 may be associated with high-risk features in CML and with a lower probability of response to IFN-α.[20]

Hypomethylating agents have been investigated in CML showing clinical efficacy, although their use has been mostly limited to advanced stages of the disease. 5-azacitidine (AZA) was used in combination with chemotherapy (e.g., etoposide, mitoxantrone) for patients with CML in transformation, with 25% to 60% responding

(i.e., back to chronic phase –BCP-). A pilot study of AZA alone in 14 patients with CML resulted in 2 responses (MDACC internal data). Decitabine has been more extensively studied in CML. It was first used for patients with CML in transformation as a single agent at a dose of 50 to 100 mg/m^2 over 6 hours every 12 hours for 5 days every 4-8 weeks[21]. Twenty-eight of 51 (55%) patients treated in accelerated phase and 18 of 64 (28%) in myeloid blast phase had a hematologic responses (CHR in 12 and 6, respectively). Cytogenetic responses were achieved in 14% and 8%, respectively. Therapy was almost universally myelosuppressive with infections occurring in 34% of patients, with 4 (3%) treatment-related deaths. Subsequently, lower doses have been used based the optimized demethylating effect with this schedule. In one study, decitabine was used at lower doses until the minimal effective dose was reached. A dose of 15 mg/m^2 daily for 10 days (i.e., 15% to 30% of the dose previously used) was well tolerated and provided the best response in a variety of myeloid malignancies.[22] This schedule has been investigated in 35 patients with CML who have failed imatinib therapy (12 chronic, 17 accelerated, 6 blastic). A hematologic response was reported in 23 (66%) patients (CHR 12, PHR 7, HI 4). Sixteen patients (46%) had a cytogenetic response (major 6, minor 10).[23] Preclinical studies have shown synergy between imatinib and decitabine.[24] [25] This combination was used in a phase II trial in patients with advanced phase CML, most of them previously treated with imatinib. Patients received decitabine 15 mg/m^2 intravenously daily, 5 days a week for 2 weeks, and imatinib 600 mg orally daily. A hematologic response was achieved in 50% and 30% of patients in accelerated and blast phase, respectively, with major cytogenetic responses in 33% and 20%, respectively.

We hypothesize that adding AZA to a TKI may improve the molecular response of patients with CML with CCyR but no MMR. If a significant number of patients convert to a CMR, we will explore whether TKI can be safely discontinued with minimal risk of relapse. Throughout the study we will measure the effect of therapy on DNA methylation in the peripheral blood and bone marrow, both global and gene-specific.

2.4. **5-azacitidine**

Azacitidine, an analog of the pyrimidine nucleoside cytidine, has effects on cell differentiation, gene expression, and deoxyribonucleic acid (DNA) synthesis and metabolism.[26] Since the early 1970s, azacitidine 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 azacitidine in the treatment of AML. Clinical studies subsequently evaluated the effects of azacitidine 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 azacitidine in patients with MDS. These studies, in addition to other supportive data, led to the approval of Vidaza® (azacitidine) in May 2004 for the treatment of MDS.[26]

Azacitidine inhibits the methylation of newly synthesized DNA by inhibiting DNA methyltransferase (DNMT).[27-29] 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.[30] It is now widely recognized that hypermethylation of DNA is frequently associated with myelodysplastic syndromes and other cancers,[31-33] such as renal,[34] melanoma,[35] breast,[36] colorectal,[37] non-small cell lung[38] and hematologic malignancies.[39] Azacitidine is believed to exert its antineoplastic effects through hypomethylation and cytotoxicity on abnormal hematopoietic cells in the bone marrow.[40-44] Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation.[30,45,46] The cytotoxic effects of azacitidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms.[40,47-49]

The cytotoxicity of azacitidine is proportional to dose and exposure time.[40,41] Although the mechanisms of cytotoxicity are complex and multifaceted, there is general agreement that incorporation of azacitidine into DNA and ribonucleic acid (RNA), and inhibition of protein synthesis, are critically important.[50] Cytotoxicity is greatest in cells that are proliferating (S phase) and metabolically active.[40] Cytotoxic effects may also be mediated through induction of the DNA damage response pathways.[49] Nonproliferating cells are relatively insensitive to azacitidine.[40]

Toxicology studies have been conducted in mice, rats, dogs, and Rhesus monkeys.[51] 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 azacitidine.[51] In single-dose studies, the lethal dose of azacitidine after intravenous (IV) administration in mice, rats, and dogs was approximately 250 mg/m^2. Repeated daily dosing appears to increase the toxicity of azacitidine.[51] The genotoxicity of azacitidine is consistent with that of other nucleoside analogs that interact with nucleic acids.[51] Likewise, similar to other agents with cytostatic properties, azacitidine was embryotoxic and reduced the reproductive performance in mice and rats.[51]

Limited azacitidine pharmacokinetic data are currently available. Based on human plasma concentrations of total radioactivity (which represents parent drug plus circulating metabolites), azacitidine is rapidly absorbed when given subcutaneously (SC), with maximum plasma concentrations found 0.5 to 2 hours after dosing.[51] Azacitidine 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 azacitidine have not been studied.[51] A single dose (75 mg/m^2) SC versus IV crossover study in 6 MDS subjects[52] 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 azacitidine 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 azacitidine 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 azacitidine. In addition, azacitidine 75 mg/m^2 was generally well-tolerated after single SC injection or IV infusion over 10 minutes.[52]

A number of studies have looked at different parenteral doses and schedules of azacitidine, finding maximum tolerated doses of up to 500 mg/m^2 when administered weekly.[53]

During the two decades between the start of the CALGB studies and the approval of azacitidine, 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.[54] Using the IWG response criteria in MDS patients, response rates were between 40% and 70% in azacitidine 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 azacitidine was 19.3 months compared with 12.9 months for the 25 patients assigned to observation.[54]

A randomized international Phase III trial (Study AZA 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.[55] Patients were randomized to azacitidine (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 azacitidine or CCR. This trial did not allow erythropoietin. Three-hundred fifty eight patients (70% male) were randomized at 79 centers to azacitidine (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 azacitidine 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. Azacitidine 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. Azacitidine 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 azacitidine arm compared to CCR: 51% vs. 26% (p<0.0001). Azacitidine was well tolerated with safety data consistent with previous reports.

Further details can be found in the azacitidine Investigator's Brochure, which contains comprehensive pharmacology, toxicology, pharmacokinetics, pharmacodynamics, metabolism, preclinical, and clinical efficacy and safety data information.[51]

3. Criteria for Patient Eligibility

3.1. **Inclusion Criteria**

3.1.1. Patients 16 years or older with Philadelphia chromosome (Ph)- or BCR/ABL-positive CML (as determined by cytogenetics, FISH, or PCR).

3.1.2. Patients must have received FDA-approved TKI therapy for at least 18 months and not have increased their dose of FDA-approved TKI in the last 6 months. Patients participating on frontline protocols 2005-0048 (nilotinib) and 2005-0422 (dasatinib) are eligible for enrollment on this study.

3.1.3. Phase II patients must be in complete cytogenetic remission.

3.1.3.1. For the phase I portion of the study, patients may be included without a complete cytogenetic remission provided they are in chronic phase

3.1.4. Phase II patients must have detectable BCR-ABL transcript levels meeting at least one of the following criteria:

3.1.4.1. Patient has never achieved a major molecular response, and transcript levels have shown in at least two consecutive measures separated by at least 1 month to have increased by any value, or

3.1.4.2. Achieved a major molecular response that has been lost with an increase in transcript levels by at least 1-log, confirmed in two consecutive analyses separated by at least 1 month, or

3.1.4.3. The patient has received therapy for at least 2 years and does not have a sustained major molecular response, or

3.1.4.4. The patient has received therapy for at least 5 years and does not have a sustained complete molecular response.

3.1.4.5. Patients included in the phase I portion of the study are eligible regardless of their level of BCR-ABL transcripts.

3.1.5. Patients must not have had a known continuous interruption of imatinib therapy of greater than 14 days or for a total of 6 weeks in the 6 months prior to enrollment.

3.1.6. Patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital.

3.1.7. ECOG performance status ≤2.

3.1.8. Adequate organ function defined as: bilirubin <2x upper limit of normal (ULN) (unless associated with Gilbert's syndrome), and ALT or AST ≤2.5x ULN.

3.1.9. ANC ≥1 $x10^9$/L and platelets ≥50 $x10^9$/L.

3.1.10. Serum creatinine < 1.8 mg/dL or creatinine clearance greater or equal than 40 cc/min as defined by the Cockcroft-Gault Equation*. Males(mL/min):(140-age)*ABW(kg) / 72*(serum creatinine(mg/dl)); Females (mL/min):0.85*(140-age)*ABW(kg) / 72*(serum creatinine (mg/dl))

3.1.11. Women of childbearing potential should be advised to avoid becoming pregnant and practice effective methods of contraception. Men should be advised not to father a child while receiving treatment with azacitidine. Azacitidine is classified as Pregnancy Category D. Females of childbearing potential: Recommendation is for 2 effective contraceptive methods during the study. Adequate forms of contraception are double-barrier methods (condoms with spermicidal jelly or foam and diaphragm with spermicidal jelly or foam), oral, depo provera, or injectable contraceptives, intrauterine devices, and tubal ligation. Male patients with female partners who are of childbearing potential: Recommendation is for male and partner to use at least 2 effective contraceptive methods, as described above, during the study.

3.1.12. Women of childbearing potential should have a pregnancy test within 7 days before initiation of study drug.

3.2. **Exclusion Criteria**

3.2.1. Patients receiving any other investigational agents

3.2.2. Patients who are pregnant or breast-feeding

3.2.3. Patients with clinically significant heart disease (NYHA Class III or IV)

3.2.4. Known or suspected hypersensitivity to azacitidine or mannitol.

3.2.5. Patients with advanced malignant hepatic tumors.

4. Treatment Plan

4.1. **TKI**: Patients will continue receiving TKI at the dose they had been receiving during the last 6 months.

4.2.**AZA:**

Phase I: patients will be treated in cohorts of 3. The starting dose will be dose level 0 (50 mg/m^2/d x 3 days subcutaneous or intravenous; Table 1).

4.2.1. At least 3 patients will be entered at each level. All patients treated at any one dose level must have been observed for at least four weeks before escalating to the next dose level. Patients who come off study prior to the first four weeks and have not experienced a DLT will be replaced.

Table 1: **Dose escalation levels**

Dose level	**AZA (mg/m^2/d x 3 days)**
-3	25 (1 day only)
-2	25 (2 days only)
-1	25
0	50
1 (Target dose)	75
2	75 (for 5 days)
3	75 (for 7 days)

4.2.2. Dose escalation will be done as follows:

Dose-limiting toxicity in	**Result**
0/3	Escalate to next level
1/3	Enter 3 more patients
≥2/3 or ≥ 2/6	MTD exceeded. This dose level will be declared the maximally administered dose. Expand previous level to include a total of 6 patients if not already accrued to this number.

4.2.3. **Definition of Dose-Limiting Toxicity (DLT)**

4.2.3.1. DLT will be defined by events that are clinically significant and possibly related to study drug occurring during the first 4 weeks (1 cycle) of therapy.

4.2.3.2. Non-hematologic DLT is defined as grade 3 or 4 toxicity (NCI common criteria, version 4.0) that is clinically significant and

considered to be at least possibly related to the study drug. Grade 3 or 4 nausea, vomiting and diarrhea will be considered DLT only if not controlled by optimal therapy.

4.2.3.3. Grade 3 biochemical abnormalities (e.g., lipase or bilirubin elevation) will only be considered DLT if accompanied by clinical consequences. Grade 3 or 4 electrolyte abnormalities will only be considered DLT if possibly related to study drug and not corrected by optimal replacement therapy.

4.2.3.4. 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 case of a normocellular bone marrow, 8 weeks with pancytopenia will be considered DLT). Anemia will not be considered for the definition of DLT.

4.2.4. If dose-level 0 exceeds MTD, subsequent patients will be treated at dose level -1. Additional cohorts at lower doses (down to dose level -4) may be included if higher doses exceed MTD. If dose level -4 exceeds MTD, the study will be terminated.

4.3. **Phase II:** The dose to be used for the phase II portion of the study is 75mg/m^2 daily for 3 days, unless MTD is defined at a lower dose level in the phase I portion of the study in which case that would become the dose to be used for the phase II portion. Other dose levels shown in Table 1 will be used for dose adjustments for toxicity or lack of improvement (as described in section 4.5.5) during therapy. For phase II portion of the study, if at any time patients need to be escalated to dose level +2 or +3, if at any time 33% or more of patients experience DLT at this dose level during the first cycle they receive at this dose, this would be considered to have exceeded MTD and that dose level should not be used any further.

4.4. Treatment with AZA (in both phase I and phase II) may be repeated every 28 days. The start of subsequent cycles may be delayed to allow for recovery of any adverse events to grade 1 or less.

4.5. **Dosing Delays/Dose Modifications**

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

4.5.2. 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.

4.5.3. **TKI:** dose modifications will be done according to institutional guidelines. The following guidelines may be used for dose adjustments:

4.5.3.1. Non-Hematologic Toxicity

- **Grade 2:** Patients with persistent grade 2 toxicity that is considered clinically significant, unresponsive to appropriate therapy, may have treatment held until the toxicity has resolved to grade ≤1. TKI may then be resumed at the same dose the patient was receiving at the time treatment was interrupted. If the grade 2 toxicity recurs, TKI may be held until the toxicity has resolved to grade 1. Treatment may then be resumed with a one dose level reduction.
- **Grade 3-4**: If a patient experiences Grade 3-4 toxicity that is considered clinically significant and related to TKI, therapy may be withheld until the toxicity has resolved to Grade ≤ 1. TKI may then be resumed with a one dose level reduction.

4.5.3.2. Hematologic Toxicity

If granulocytes are $<0.5 \times 10^9$/L or platelets are $<40 \times 10^9$/L, hold therapy until granulocytes are above 10^9/L and platelets are above 60×10^9/L, then resume therapy.

If recovery takes more than 2 weeks, resume TKI at 1 dose level reduction from the dose the patient was receiving at the time therapy was interrupted.

If recovery takes less than 2 weeks, resume TKI at the same dose the patient was receiving at the time treatment was interrupted. If myelosuppression recurs, resume TKI at one dose level reduction from the dose the patient was receiving at the time the treatment was interrupted.

If a similar degree of toxicity returns, a further dose reduction by one dose level can be performed, using the above procedures.

4.5.3.3. The usual dose levels for dose adjustments are as follows:

Imatinib (mg/d)	Dasatinib (mg/d)	Nilotinib (mg/d)
800	140	800
600	100	600
400	70	400
300	50	300
200	40	200
100	20	150

4.5.3.3.1. The maximum allowable daily doses of TKI are: dasatinib 140 mg/d, nilotinib 800 mg (usually as 400 mg BID), and imatinib 800 mg.

4.5.3.4. Modifications of dose schedules other than the above will be allowed within the following guidelines:

- Further dose reductions can be made to keep toxicity grade ≤2.
- Dose adjustments by more than 1 dose level at a time can be considered when judged in the best interest of the patient (e.g., neutropenia with sepsis, bleeding requiring platelet transfusions) when toxicity has resolved.
- A patient who has had a dose reduction because of any reason may have their dose re-escalated provided the patient has remained free of toxicity requiring dose adjustments as defined above for at least 1 month. Escalation will be made by 1 dose-level increments only, and not more frequent than every month.

4.5.4. **AZA**: dose modifications for toxicity will be done according to the following guidelines:

4.5.4.1. Non-Hematologic Toxicity

4.5.4.1.1. **Persistent Grade 2:** Patients with persistent grade 2 toxicity that is considered clinically significant, unresponsive to appropriate therapy, may have treatment held until the toxicity has resolved to grade ≤1. Therapy may then be resumed at the same dose the patient was receiving at the time treatment was interrupted. If the grade 2 toxicity recurs, therapy may be held until the toxicity has resolved to grade 1. Treatment may then be resumed with a one dose level reduction.

4.5.4.2. **Grade 3-4**: If a patient experiences Grade 3-4 toxicity that is considered clinically significant and possibly related to AZA, therapy must be withheld until the toxicity has resolved to Grade ≤1. Treatment may then be resumed with a one dose level reduction. If toxicity recurs, additional dose reductions may be implemented according following the same general guidelines.

4.5.4.3. If unexplained reductions in serum bicarbonate <20mEq/L occur, the dosage should be reduced by 50% on the next course. Similarly, if unexplained elevations of BUN or serum creatinine occur, the next cycle should be delayed until values return to normal or baseline and the dose should be reduced by 50% on the next treatment course.

4.5.4.4. Hematologic Toxicity

4.5.4.4.1. If neutrophils are <0.5 x 10^9/L and/or platelets are <40 x x10^9/L, hold therapy until granulocytes are above 1 x10^9/L and platelets are above 60 x10^9/L, then resume therapy.

4.5.4.4.2. If recovery takes more than 2 weeks, resume therapy at 1 dose level reduction from the dose the patient was receiving at the time therapy was interrupted.

4.5.4.4.3. If recovery takes less than 2 weeks, resume therapy at the same dose the patient was receiving at the time treatment was interrupted. If myelosuppression recurs, resume therapy at one dose level reduction from the dose the patient was receiving at the time the treatment was interrupted.

4.5.4.4.4. If a similar degree of toxicity returns, a further dose reduction by one dose level can be performed, using the above procedures.

4.5.4.4.5. 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 above.

4.5.4.4.6. There will be no making up for missed doses.

4.5.4.5. Modifications of dose schedules other than the above will be allowed within the following guidelines:

4.5.4.5.1. Further dose reductions can be made to keep clinically significant, AZA-related toxicity grade ≤2. However, the lowest acceptable dose level is -4.

4.5.4.5.2. Dose adjustments by more than 1 dose level at a time can be considered when judged in the best interest of the patient (e.g., neutropenia with sepsis, bleeding requiring platelet transfusions) when toxicity has resolved. The reason for this reduction will be discussed with the PI and the drug supplier and documented in the medical record.

4.5.4.5.3. A patient who has had a dose reduction because of any of the reasons mentioned above may have their dose re-escalated provided the patient has remained free of toxicity

requiring dose adjustments as defined above for at least 1 month. Escalation will be made by 1 dose-level increments only, and not more frequent than every month.

4.5.5. **Dose Escalation**: Patients that do not achieve an improvement in molecular response after 3 months (eg., from major to complete or from non-major to major) and have experienced no grade ≥3 toxicity may have the dose of AZA escalated by one dose level. Patients who have had a prior dose reduction of AZA and who have no toxicity for at least 1 month of therapy may increase the dose of AZA following the guidelines mentioned above. Dose escalation of the TKI is also allowed according to institutional guidelines. However, concomitant dose escalation of the two agents is not allowed. Patients who have had dose escalation of TKI cannot have dose escalation of AZA within 2 months of this escalation.

4.5.6. Occasional missed doses of TKI will not be considered a deviation. Missed dosed of ≥ 2 weeks without adequate justification will be considered a protocol deviation.

4.5.7. **Duration of Therapy:** Patients may receive therapy for six cycles. After completion of the 6^{th} cycle, patients may continue therapy if in the opinion of the investigator there is clinical benefit provided there has been no grade 3 or higher non-hematologic toxicity attributable to AZA at the doses being administered.

4.5.7.1. Patients who achieve a major or complete molecular remission can hold therapy with AZA and/or TKI and be monitored with PCR. If there is reappearance of transcripts detectable by PCR, therapy with one or both agents can be resumed at the doses being administered at the time treatment was interrupted.

4.5.8. **Concomitant Medications**

4.5.8.1. Patients may not receive any other treatment for CML while on study. This includes the use of hydroxyurea and anagrelide.

4.5.8.2. The use of other medications for management of comorbidities or adverse events is allowed as clinically indicated.

5. Evaluation During Study

5.1. **Pretreatment Evaluation** (within 7 days from treatment start, unless otherwise specified)

5.1.1. A complete history and physical examination including performance status.

5.1.2. CBC, platelet count and differential (differential not required if WBC < 0.5 $x10^9$/L), total bilirubin, SGPT (or SGOT), and creatinine within 1 week.

5.1.3. Bone marrow aspirate for morphology and cytogenetics or FISH (if not done within 3 months).

5.1.4. Pregnancy test (blood or urine) for female patients of childbearing potential within 7 days before initiation of study drug dosing.

5.1.5. Peripheral blood and/or bone marrow for quantitative PCR (QPCR) (if not done within 3 months).

5.1.6. Peripheral blood and/or bone marrow for correlative studies (optional – Appendix A, B & C). Not all optional samples may be collected.

5.2. **Evaluation During Study**

5.2.1. Physical exam and evaluation of toxicity (clinic visit or telephone interview):

5.2.1.1. Phase I portion: every 2 weeks for the first month, then every month for the first 3 months, then every 3 months (±1 month) until month 6, then every 6 to 12 months.

5.2.1.2. Phase 2 portion: every 3 months (±1 month) until month 6, then every 6 to 12 months.

5.2.2. CBC, platelet, differential every 1-2 weeks for 8 weeks, then at the start of every cycle. Differential not required if WBC $\leq 0.5 \times 10^9$/L.

5.2.3. Bone marrow aspirate with cytogenetics or FISH every 3-6 months in year 1, then as clinically indicated.

5.2.4. Total bilirubin, SGPT or SGOT, and creatinine every 1-2 weeks for 8 weeks, then before each cycle.

5.2.5. Quantitative PCR (peripheral blood and/or bone marrow) before each cycle for 3 cycles, then every 3-6 cycles until one year, then every 6-12 cycles.

5.2.6. Peripheral blood and bone marrow for correlative studies every 3-6 months in year 1, then every 6-12 months. Methylation studies will also be done on days 5, 10 and 28 (all ± 3 days) of the first cycle. Not all optional samples may be collected. (Optional – Appendix A, B & C).

6. Criteria for Response

6.1. A favorable response for the primary endpoint is defined as a greater than a one-log reduction of BCR-ABL transcript levels from the baseline level at the time vaccination was initiated, or a disappearance of BCR-ABL transcripts (i.e., complete molecular response).

7. Criteria for Removal from Study

7.1. The patient receives CML-directed therapy other than TKI and AZA.

7.2. The patient develops disease progression defined as confirmed loss of CCyR, confirmed loss of CHR or transformation to accelerated or blast phase.

7.3. The dose of TKI is increased or new therapies for their leukemia are added.

7.4. The patient has a continuous interruption of imatinib therapy for greater than 6 weeks.

7.5. The patient withdraws consent.

7.6. The treating physician considers it in the best interest of the patient.

7.7. Pregnancy or suspected pregnancy

8. Adverse Event Reporting

8.1. 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.

8.2. Adverse Events (AEs) will be evaluated according to the latest CTC version and documented in medical record. Only unexpected AEs will be recorded in the Case Report Form (CRF). Expected events during leukemia therapy are:

8.2.1. **Myelosuppression Related Events (due to disease or leukemia therapy)**

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

8.2.1.2. epistaxis or bleeding except for catastrophic CNS or pulmonary hemorrhage

8.2.1.3. anemia, neutropenia, lymphopenia, thrombocytopenia, leukopenia

8.2.2. **Disease Related Events**

8.2.2.1. symptoms associated with anemia

8.2.2.2. fatigue

8.2.2.2.1. weakness

8.2.2.2.2. shortness of breath

8.2.2.3. electrolyte abnormalities (sodium, potassium, bicarbonate, CO2, magnesium)

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

8.2.2.5. coagulation abnormalities

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

8.2.2.7. alopecia

8.2.2.8. bone, joint, or muscle pain

8.2.2.9. liver function test abnormalities associated with infection or disease progression

8.2.2.10. disease progression

8.2.2.11. abnormal hematologic values

8.2.3. **General Therapy Related Events**

8.2.3.1. catheter related events

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

8.2.3.3. rash related to antibiotic use

8.2.3.4. Hospitalization for the management of any of the above expected events

8.3. 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.

8.4. **Serious Adverse Event Reporting (SAE)**

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 IND 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, 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.

8.5. **Annual Reports**

If the FDA has granted an IND number, it is a requirement of 21 CFR 312.33, that an annual report is provided to the FDA within 60-days of the IND anniversary date. 21 CRF 312.33 provides the data elements that are to be submitted in the report. The Annual Report should be filed in the study's Regulatory Binder, and a copy provided to Celgene Corporation as a supporter of this study as follows.

Celgene Corporation

Attn: Medical Development

86 Morris Avenue

Summit, NJ 07901

Tel: (908) 673-9000

8.6. **Pregnancies:**

Pregnancy of a female subject or the female partner of a male subject occurring while the subject is on azacitidine or within 28 days after the subject's last dose of Azacitidine are considered expedited reportable events. If the subject is on Azacitidine, it is to be discontinued immediately. The pregnancy must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the pregnancy by facsimile using the SAE Form.

The Investigator will follow the pregnant female until completion of the pregnancy, and must notify Celgene Drug Safety of the outcome as specified below. The Investigator will provide this information as a follow-up to the initial SAE.

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for Expedited Reporting of SAEs to Celgene (i.e., report the event to Celgene Drug Safety by facsimile within 24 hours of the Investigator's knowledge of the event).

Any suspected fetal exposure to Azacitidine must be reported to Celgene within 24 hours of being made aware of the event. The pregnant female should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling.

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator suspects is related to the in utero exposure to Azacitidine should also be reported.

In the case of a live "normal" birth, Celgene Drug Safety should be advised as soon as the information is available.

Celgene Drug Safety Contact Information:

Celgene Corporation

Drug Safety

86 Morris Avenue

Summit, N.J. 07901

Toll Free: (800)-640-7854

Phone: (908) 673-9667

Fax: (908) 673-9115

e-mail: drugsafety@celgene.com

9. Statistical Considerations

9.1. This is a single arm, open label, phase I-II trial to evaluate the toxicity and efficacy of the combination of AZA and a TKI in CML patients in complete cytogenetic remission with minimal residual disease. A total of 48 patients will be accrued at a rate of 1 to 2 patients per month.

9.2. **Phase I**

The primary objective of phase I part is to determine the maximum tolerated dose (MTD) of AZA; and the primary endpoint is the occurrence of dose-limiting toxicity (DLT: section 4.3.3) within the first course of treatment. Standard 3+3 design will be used. The starting dose is 50 mg/m^2/d x 3 days (Table 1).

Table 1: Dose escalation levels	
Dose level	AZA (mg/m^2/d x 3 days)
-1	25
0	50
1	75

9.2.1. The 3+3 algorithm is described below:

9.2.1.1. Three patients will be enrolled at the starting dose level

9.2.1.2. If 0/3 patient experiences DLT at a given level, enroll three patients to the next higher dose level

9.2.1.3. If 1/3 patient experiences DLT at a given level, enroll additional three patients to the same dose level

9.2.1.4. If 2/3 or 2/6 patients experience DLT at a given level, the MTD has been exceeded and dose escalation will be stopped. Up to three additional patients will be enrolled at the next lower dose level if only 3 patients were treated at that level.

9.2.2. MTD is defined as the highest dose level in which 6 patients have been treated and one or fewer patient experiences DLT.

The MTD identified in phase I part will be recommended for phase II. If MTD is not determined, the highest AZA level of 75 mg/m^2/d x 3 days will be used as the recommended phase II dose.

A maximum of 18 patients will be accrued for phase I part. The data from the 6 patients treated at the recommended phase II dose level (MTD or 75 mg/m^2/d x 3 days if MTD is not determined) will be used in phase II part.

9.3. **Phase II**

The primary endpoint for phase II portion is the decrease of transcript levels by at least one log (or undetectable transcript level) within 12 months. Including the 6 patients treated at the recommended phase II dose level in the phase I part, a total of 36 patients will be accrued.

9.3.1. Efficacy Monitoring

Since the efficacy of the drug will be assessed over a long period of time (12 months) and the accrual rate is relatively fast (1-2 patients per month), no formal futility monitoring rule will be applied. However, the PI and the study committee will meet regularly, and the study will be stopped early if consensus has been reached on any clear indications of poor efficacy of the drug.

9.3.2. Toxicity Monitoring

The method of Thall, Simon, and Estey will be used to perform interim safety monitoring [1]. The safety monitoring of the trial will be initiated after the first 6 patients have been evaluated, and then monitored continuously. The monitoring rule for toxicity is $P(\theta_E>0.20|data)>0.90$, where θ_E is the proportion of any DLT (as defined in section 4.3.3), and is assumed to follow a non-informative prior of Beta (0.4, 1.6). Namely, the trial will be terminated if at any time during the study there is a more than 90% chance that the average rate of DLT is more than 20%. The stopping boundaries of the trial are ≥3out of 6, ≥4 out of 7, ≥5 out of 10, ≥6 out of 14, ≥7 out of 18, ≥8 out of 22, ≥9 out of 26, ≥10 out of 30, ≥11 out of 34 patients experienced DLT.

Table 2: Operating characteristics of Safety Monitoring (based on 10000 simulations)

True Probability	Stop Probability	Trial Sample Size Percentile		
		25%	50%	75%
0.10	0.02	36	36	36
0.20	0.25	36	36	36
0.30	0.69	6	17	36
0.40	0.96	6	8	16

All calculations were performed using Multc99.

9.4. Statistical Methods

Data analysis will be performed using SAS or S-plus, whichever is appropriate. All patients who receive at least one dose of therapy will be included in the primary efficacy and toxicity analysis. Patients who do not have molecular response and

withdraw within 12-months after initiation of treatment will be counted as failure in the primary efficacy analysis.

The rate of molecular response will be estimated with 95% confidence interval. The null hypothesis is that the response rate is 5%, and alternative hypothesis is that the response rate is 20%. If a 20% response is reached, the study would be considered positive. Under this setting, 36 patients can provide a power of 87% for a one-sided test of the above hypothesis at significance level alpha=5%.

10. Protocol Amendments, Deviations and Regulatory

Any amendment to this protocol must be agreed to by the Principal Investigator and by MD Anderson Cancer Center and by Celgene. Amendments should only be submitted to IRB after consideration of M. D. Anderson Cancer Center, Celgene review. Written verification of IRB approval will be obtained before any amendment is implemented.

10.1. **Protocol Deviations**

When an emergency occurs that requires a deviation from the protocol for a subject, a deviation will be made only for that subject. A decision will be made as soon as possible to determine whether or not the subject (for whom the deviation from protocol was effected) is to continue in the study. The subject's medical records will completely describe the deviation from the protocol and state the reasons for such deviation. In addition, the Investigator will notify the IRB in writing of such deviation from protocol.

10.2. **Investigator Responsibilities**

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations.

Investigators must enter study data into MDACC's PDMS. The Investigator will permit study-related monitoring visits and audits by Celgene or its representatives, IRB/EC review, and regulatory inspection(s) (e.g., FDA, EMEA, TPP), providing direct access to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

The Investigator, or a designated member of the Investigator's staff, must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the subject's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The data collection must be completed prior to each visit and be made available to the Celgene representative so that the accuracy and completeness may be checked.

10.3. **Institutional Review Board/Ethics Committee Approval**

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB/EC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The Investigator will be responsible for preparing documents for submission to the relevant IRB/EC and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study.

The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.

Any amendments to the protocol after receipt of IRB/EC approval must be submitted by the Investigator to the IRB/EC for approval. The Investigator is also responsible for notifying the IRB/EC of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB/EC prior to use.

10.4. Informed Consent

The Investigator must obtain informed consent of a subject or his/her designee prior to any study related procedures as per GCPs as set forth in the CFR and ICH guidelines.

Documentation that informed consent occurred prior to the subject's entry into the study and the informed consent process should be recorded in the subject's source documents.

10.5. Study Records Requirements

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, that is copies of CRFs and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; subject files; and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study; documents regarding subject treatment and study drug accountability; original signed informed consents, etc.]) be

retained by the Investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). The Investigator agrees to adhere to the document/records retention procedures by signing the protocol.

10.6. Premature Discontinuation of Study

The responsible local clinical Investigator as well as Celgene has the right to discontinue this study at any time for reasonable medical or administrative reasons in any single center. Possible reasons for termination of the study could be but are not limited to:

10.6.1. Unsatisfactory enrollment with respect to quantity or quality.

10.6.2. Inaccurate or incomplete data collection.

10.6.3. Falsification of records.

10.6.4. Failure to adhere to the study protocol

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