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Protocol Page

A RANDOMIZED PHASE II STUDY OF TWO SCHEDULES OF DECITABINE FOR FRONTLINE THERAPY OF OLDER OR UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)
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Study Chair:	Farhad Ravandi-Kashani
Additional Contact:	Andrea L. Booker Priscilla D. Cardenas Leukemia Protocol Review Group
Department:	Leukemia
Phone:	713-745-0394
Unit:	428
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A RANDOMIZED PHASE II STUDY OF TWO SCHEDULES OF DECITABINE FOR FRONTLINE THERAPY OF OLDER OR UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA

Principal Investigator: Farhad Ravandi-Kashani, M.D.

Biostatistician: Xuelin Huang, Ph.D.

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A RANDOMIZED PHASE II STUDY OF TWO SCHEDULES OF DECITABINE FOR FRONTLINE THERAPY OF OLDER OR UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA

1.0 Objectives

1.1 **Primary**: Compare the response rates of two schedules of decitabine in patients with AML 60 and older than 60 and/or unfit for standard cytotoxic chemotherapy.

1.2 **Secondary**: Compare response durations, survivals and side effects of the two schedules.

1.3 **Tertiary**: To examine the correlation of a number of biological and pharmacodynamics correlates with response to therapy including global and gene-specific methylation, specific somatic gene mutations, micro-RNA, and immune effector function

2.0 Background

2.1 The Disease – Acute Myeloid Leukemia in the elderly

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults in the US, with an incidence of approximately 12,000 per year.[1] The median age at diagnosis is 65-70 and the incidence rises with increasing age with the age-adjusted incidence being 17.6 per 100,000 and 1.8 per 100,000 in those older and younger than 65, respectively. This problem is not unique to the US with an increase in the incidence of AML in the older population worldwide, likely due to an overall increase in life-expectancy, increased application and success of chemotherapeutic agents and radiation therapy in cancer patients, and perhaps an overall increase in environmental toxin exposure as a result of industrialization.

2.1.1 Attitudes towards treatment of older patients

Despite the high incidence of AML in the older population, until recently, many of the trials in this disease had been conducted in the younger population and much of the available data was inapplicable to them. This reflected reluctance by both patients and physicians to expose the older patients to the toxic effects of anti-leukemic therapy. Such under-representation of patients 65 years and older in cancer treatment trials has been well documented but is probably more of an issue in acute leukemias where the chemotherapeutic regimens are generally more intensive than in other tumors.[2] Furthermore, clinical trials conducted in the older population have been generally biased due to the exclusion of patients with poor performance status and co-morbid medical conditions. Using the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare database, Menzin and colleagues retrospectively evaluated the outcomes of approximately 3500 elderly patients with AML.[3,4] They reported that only about a third of the patients received induction chemotherapy, ranging from 7% of patients ≥ 85 years of age to 49% of patients 65-74 years of age. Treatment rates increased during the decade of analysis, ranging from 29% to 38% of patients diagnosed in

1991 and 1999 respectively but the increased rates were confined to younger patients (those 65-74 vs. 75-84 years of age).[4] Median survival across all study population was 2.4 months with fewer than 7% of patients alive at 2 years further emphasizing the dismal outcome of these patients in the community with current strategies. However, the authors also demonstrated that patients who did receive therapy benefited from a prolongation of survival, be it modest.[3]

Clearly the decision making process in treating elderly patients with AML has been highly influenced by the attitudes of patients and their physicians and their expectations of success. Juliusson and colleagues, using the Swedish Leukemia Registry data, retrospectively evaluated the outcomes of 506 patients aged 70-79 with AML (Acute promyelocytic leukemia-APL excluded) from 6 Swedish health regions with known differing attitudes towards remission induction.[5] Although the 5-year survival of the overall 70-79 year old population in these regions was similar, the survival of 70-79 year-old AML patients was significantly better in regions were more elderly patients were judged eligible for remission induction.[5]

In a more recent study, the same investigators and using data from the same registry reported an improvement in early death rates and long-term survival among elderly patients who received induction chemotherapy as compared to those who received palliation only.[6] Furthermore, long term survivors were found in the group given intensive therapy despite poor initial performance status.[6] This has also been reported by a number of other studies that have compared chemotherapy (intensive or non-intensive) to palliation.[7,8] Lowenberg and colleagues randomized 60 patients with AML aged 65 or older to receive either immediate intensive induction chemotherapy or palliative treatment with only mild cytoreductive chemotherapy only for relief of progressive disease.[7] They demonstrated a higher complete remission (CR) rate (58% vs. 0%), and a higher survival duration (21 vs. 11 weeks, p=0.15) in patients treated more intensively.[7] Results from the non-intensive chemotherapy arm of the AML14 trial showed that median survival significantly improved with low-dose cytarabine (20 mg twice daily for 10 days, every 4 to 6 weeks) compared with hydroxyurea.[8] CR occurred in 1 of 92 (1%) patients in the hydroxyurea treatment arm and in 15 of 92 (17%) patients in the low-dose cytarabine arm. Benefits were limited to patients who had intermediate-risk karyotype. In another earlier study, Tilly and colleagues randomized 87 patients 65 years and older to low dose cytarabine (20 mg/m^2 daily for 21 days) or intensive chemotherapy with cytarabine and an anthracycline.[9] Overall survival and CR duration was similar for the two groups with significantly higher CRs as well as significantly higher early death and severe infections in the in the intensively treated group.[9]. Clearly and considering the results from the hitherto mentioned studies, this population benefits at least modestly from receiving chemotherapy as opposed to palliation only. The decision regarding the intensity of the chemotherapy administered remains one of the most debated issues in AML therapy. It is also clear that although age per se is an important predictor of the outcome, other factors (covariates) influence it. The heterogeneity of patients labeled “elderly” is clearly masked by terms commonly used to describe this population such as “ineligible to undergo intensive chemotherapy”. It is also worth noting here that the estimations of response to intensive therapy may be highly divergent between the patients and their treating physicians, further complicating the process of decision-making.[10]

2.1.2 Predictors of outcome in elderly AML

Age per se has been repeatedly shown to be associated with an inferior outcome when considering fairly uniformly treated populations of patients (Figure 1a).[11,12] In a study by the German AML co-operative group, 4-year survival in those younger and older than 60 years was 37% vs. 16% ($p<0.001$).[12] It can be argued that age should be considered as a continuum with no clear demarcating age above and below which patient is considered elderly. However, for practical purposes, age ≥60 years is a commonly accepted criterion for defining elderly. Appelbaum and colleagues analyzed data from 5 Southwest Oncology Group (SWOG) clinical trials which included 968 patients.[11] Increasing age was associated with less favorable cytogenetics, poorer performance status scores, lower white blood cell counts, and a lower percentage of marrow blasts.[11] Increasing age was also associated with a higher incidence of early death after induction therapy, a lower CR rate, and shorter survival.[11] However, among the older population, those with a poorer performance status at presentation had a very high early mortality an indication of the interaction between covariates.

Other factors that are clearly important predictors of outcome include patient-related factors such as organ function, and presence of uncontrolled infection, and leukemia-related factors such as biological determinants of resistance to chemotherapy. Cytogenetics has been clearly established as one of the most important predictors of outcome in AML.[13-15 16] Although the overall outcome of older patients with AML is inferior to younger adults, survival is generally improved for patients with favorable and intermediate cytogenetic aberrations compared to those with adverse karyotype. Recent data confirm the importance of pre-treatment karyotype on the outcome of older patients.[17-20] In a report examining the prognostic factors in older patients treated on the MRC AML11 and AML14 trials, the survival was very much dependent on the cytogenetic risk. In another study by the German-Austrian AML study group high risk cytogenetics and age above 70 years were independent prognostic factors affecting overall survival.[19] Stratification using these parameters identified a large subgroup (55%) of patients with very poor outcome despite intensive chemotherapy. Whether pre-treatment cytogenetics should be factored in the choice of therapy in older AML patients remains a subject of debate. As typically the result of this analysis may not be available for up to a week, it can be argued that cytogenetics should not influence the initial decision making process in selecting therapy. However, a recent study by Sekeres and colleagues demonstrated that accounting for other covariates available at the time of diagnosis, in patients older than 60 years and with white blood cell (WBC) less than 50×10^9/L, time from diagnosis to initiation of therapy did not affect CR rate or overall survival suggesting that this population may not be harmed from a delay to obtain the results of additional testing.[21] Others have also recommended waiting until the cytogenetic results are available.[22] Obviously, this is highly dependent on the availability of treatment strategies that are clearly superior to standard in particular cytogenetic subgroups or the recognition that patients with specific characteristics such as those described by Malfuson and colleagues are unlikely to benefit from standard induction regimens.[22]

More recently, it has been reported that younger patients with normal karyotype can be further characterized by the absence or presence of specific molecular aberrations that can affect their outcome.[23] Few studies have examined the role of these molecular abnormalities and their influence on the outcome of elderly patients with AML. However, in a recent study, the investigators from the Cancer and Leukemia Group B (CALGB) reported that mutations in the nucleophosmin (NPM1) gene were associated with a better prognosis in cytogenetically

normal older patients and in particular in those ≥ 70 years.[24] Other predictors of a worse outcome in the elderly have been identified. A higher incidence of intrinsic drug resistance in the leukemic blasts, mediated by expression of multidrug resistance glycoprotein MDR1 (and other efflux pumps that actively extrude chemotherapeutic agents from leukemic cells), has been reported in older patients with AML and has been associated with a lower CR rate and increased resistance.[25] Similarly, presence of antecedent hematological disorders such as myelodysplastic syndromes (MDS) and myeloproliferative disorders (MPD) as well as co-morbidity are well-known predictors of a poorer outcome.[26]

2.1.3 Choice of therapy

Therefore, although the outcome of treatment for the older patients with AML is generally inferior to that in the younger patients, it is possible to identify subgroups of patients based on their disease biology and clinical condition who are likely to fare better with the more intensive standard regimens. Previous studies of traditional chemotherapy in selected older patients have reported relatively high CR rates.[27,28] Indeed, it may even be possible to further Intensify the treatment in selected older patients as was demonstrated in the study by Lowenberg and colleague in which among patients 60-65 years a higher dose of daunorubicin at induction (90 mg/m^2 vs. 45 mg/m^2) was associated with a higher CR rate (73% vs. 51%), event-free survival (29% vs. 14%), and overall survival (38% vs. 23%).[29] In the study by Pautas and colleagues using either daunorubicin 80 mg/m^2 daily x 3 or idarubicin 12 mg/m^2 daily for 3 or 4 days in addition to standard dose cytarabine in patients aged 50 to 70 years, the overall CR rate was 77%.[28] However, in the majority of these studies there is a selection against patients with poor performance status and poor organ function and, in some, patients with history of antecedent hematological disorder such as MDS or MPD are excluded. in general, strategies to improve the efficacy of the traditional cytarabine plus anthracycline regimens have largely benefited the younger patients with less adverse characteristics.[29,30]

A number of risk scores have been developed that can be used to identify patients less likely to benefit from the traditional cytarabine containing induction regimens.[17,18,22] Kantarjian and colleagues were able to divide 998 patients with AML or high-risk MDS ≥ 65 years to favorable, unfavorable and intermediate subgroups with different expectations of CR, 8-week mortality, and survival.[17] It is potentially possible to use these scoring systems to select patients with a low expectation of CR and a high likelihood of early mortality with such regimens for clinical trials of investigational agents designed against specific molecular targets or drugs thought to be more tolerable and/or convenient.[31]

2.2 The Treatment - Decitabine (5 aza-2'deoxycytidine, Decitabine)

Decitabine is a deoxycytidine analog which is phosphorylated to its nucleotide and incorporated into DNA. Once incorporated, it covalently binds to DNA-methyltransferases and traps the enzyme thus acting as an irreversible inhibitor of DNA-methyltransferase. Decitabine produces marked DNA hypomethylation (superior to azacytidine in this effect) via inhibition of DNA methyltransferase.[32-34] At high doses, decitabine appears to cause DNA synthesis arrest due to the formation of a DNA/DNA-methyltransferase adducts, which results in cytotoxicity and apoptosis. At low doses, minimal cytotoxicity is observed, and treated cells exhibit marked reduction in DNA-methyltransferases activity, reduced overall and gene-

specific DNA methylation and reactivation of silenced genes. This is associated with tumor suppressor gene activation, induction of cellular differentiation, and inhibition of clonogenic growth of leukemic progenitors.

2.2.1. Clinical Experience with Decitabine at standard doses at MD Anderson

A phase 2 trial of Decitabine in accelerated phase (CML-AP) and blastic phase CML (CML-BP) was completed at MD Anderson. Initially, patients were treated at 100 mg/m^2 q12h for 5 days (1,000 mg/m^2 per course) but the dose had to be reduced initially by 25% and eventually by 50% because of prolonged myelosuppression. In CML-BP, a total of 42 patients were treated, with a complete hematologic response seen in 5%, partial hematologic response in 5% and hematologic improvement in 19%, for a total response rate of 29%. In CML-AP, a total of 39 patients were treated. CHR was seen in 18%, PHR in 38% and HI in 8%, for a response rate of 62%. Unique features of decitabine in this setting were:(1) a slow pattern of reduction of blasts, (2) a rapid rise in platelets following therapy, and (3) occasional responses late into therapy (after 2 cycles). These suggested that responses to decitabine might involve a differentiation component, similar to what was observed with other differentiating agents in leukemia. A subset analysis of older (>50 years) patients in CML-BP showed that decitabine therapy resulted in better survival than historical controls treated with combination chemotherapy.[35,36]

2.2.2 Clinical Experience with low-dose Decitabine at MD Anderson

Based on in-vitro data suggesting greater hypomethylating activity at lower doses, a phase I biological study of decitabine was initiated at MD Anderson. To maximize the hypomethylating effects of decitabine, multiple low dose schedules in patients with relapsed/refractory myeloid malignancies were tried. Initially, patients were treated at 5 mg/m^2 IV over 1 hour daily for 10 days (dose 30 fold lower than the reported MTD). The dose was then escalated to 10, 15 and 20 mg/m^2 daily for 10 days. Finally, a group of patients received 15 mg/m^2 daily for 15 days then 20 days. A total of 48 patients were enrolled on the study. Responses were seen at all dose levels, but 15 mg/m^2 appeared to induce the most responses, with no further benefit for increasing the dose or duration of administration. There were 9 complete remissions (CR 18%) and 7 partial remissions (14%), for a response rate of 32%. Responses were seen in refractory/relapsed AML (8/37=22%), MDS (4/7=57%), and CML (4/5=80%). In some patients who responded, there was a gradual diminution of blasts over 2-4 weeks, and eventual recovery of normal hematopoiesis at 5-6 weeks, suggesting a non-cytotoxic mode of action for this regimen. Response duration ranged from 2 months to 10+ months. DNA methylation studies are ongoing, but preliminary data suggests that hypomethylation of Alu repeats was associated with response. Therefore low-dose decitabine is an effective agent in myeloid malignancies, and appears to induce remissions in part through demethylation rather than cytotoxicity. The effective low dose administration schedule derived from this phase I study is 15 mg/m^2 IV over 1 hour daily for 10 days.[37] An ongoing study of low dose decitabine in imatinib resistant CML is confirming the good response rate of this regimen. However, in patients in chronic phase CML, grade III-IV

myelosuppression is universal at the decitabine dose of 15 mg/m^2 daily x 10. There were no other non-hematologic serious side-effects attributable to decitabine in the first 18 patients treated. Therefore, in the current study in MDS we propose to reduce the total dose per course from 150 mg/m^2 to 100 mg/m^2 to avoid excessive myelosuppressive complications.

2.2.3 Decitabine in Myelodysplastic Syndrome (MDS)

The standard for MDS therapy is hypomethylating agents, decitabine and azacytidine. The experience with 5-azacytidine indicated this to be the first non-transplant modality to affect MDS prognosis.[38] Decitabine is a hypomethylating agent similar to 5-azacytidine and is more effective in vitro. Intensive chemotherapy is associated with CR rates of 40% to 60% induction mortality rates of 10% to 40% and no improvement in MDS survival.[38]

Azacytidine, another hypomethylating agent, was given subcutaneously to 99 patients with MDS and compared with observation (n=92); 67% had advanced-stage disease by FAB. The response rate on the treatment arm was 60%: 7% CR and 16% PR, 37% HI. This was compared to 7% HI with observation ($p<0.001$). The median time to leukemic transformation or death was 21 months on treatment and 13 months on observation ($p=0.007$). Median survival was 20 months for azacytidine and 14 months for the controls difference ($p=0.1$). Quality of life measures were higher for azacytidine. Azacytidine was relatively well tolerated, although side effects included cytopenias and one treatment-related death. The results of this study appear to confirm a role for hypomethylating agents in the treatment of MDS.[38]

Several studies have shown the activity of decitabine in MDS. Zagonel et al. reported on 10 patients with advanced MDS treated with decitabine at 45-50 mg/m^2/day administered for three days. The response rate was 50% (CR and PR). The median CR duration was 11 months (10-14+ months), and the median number of courses to CR was 2.[39] Serious infections occurred in 40%, marrow hypoplasia in 50%.[39]

Wijerman et al. reported on 66 patients treated with decitabine (16 intermediate-1, 25 intermediate-2, and 25 high risk). Their median age was 68 years. Decitabine was administered at 45 mg/m^2/day given as a four-hour infusion every eight hours for three days. The response rate was 49%: 20% CR, 5% PR, and 24% improvement. Median response rate was 39 weeks. Median survival was 15 months. Normalization of abnormal karyotypes was seen. Infectious episodes were common (27% fever, 20% infection, and 11% sepsis).[40,41]

Wijermans et al recently updated the results of 169 patients with MDS treated on multiple studies with decitabine 45-50 mg/m^2 IV (continuous infusion, or over 4 hours Q 8 hours) daily x 3 (135-150 mg/ m^2 per course) every 4-8 weeks.[42] The CR rate was 20%, the overall response rate was 50%, the induction mortality was 8%. The median response duration was 9 months; the median survival was 15 months. There was no difference in response rate by risk group.

In a pivotal study, a total of 170 patients with MDS were randomized to receive either decitabine at a dose of 15 mg/m^2 given intravenously over 3 hours every 8 hours for 3 days (at

a dose of 135 mg/ m^2 per course) and repeated every 6 weeks, or best supportive care.[43] Response was assessed using the International Working Group criteria and required that response criteria be met for at least 8 weeks. Patients who were treated with decitabine achieved a significantly higher overall response rate (17%), including 9% complete responses, compared with supportive care (0%) (P < .001). An additional 12 patients who were treated with decitabine (13%) achieved hematologic improvement. Responses were durable (median, 10.3 mos) and were associated with transfusion independence. Patients treated with decitabine had a trend toward a longer median time to acute myelogenous leukemia (AML) progression or death compared with patients who received supportive care alone (all patients, 12.1 mos vs. 7.8 mos [P = 0.16]; those with International Prognostic Scoring System intermediate-2/high-risk disease, 12.0 mos vs. 6.8 mos [P = 0.03]; those with de novo disease, 12.6 mos vs. 9.4 mos [P = 0.04]; and treatment-naive patients, 12.3 mos vs. 7.3 mos [P = 0.08]). Decitabine was found to be clinically effective in the treatment of patients with MDS, provided durable responses, and improved time to AML transformation or death. The duration of decitabine therapy may improve these results further. This study led to the approval of decitabine for patients with MDS.[43]

In a more recent trial, we investigated the clinical and pharmacodynamic results of different dose schedules of decitabine.[44] Adults with advanced MDS or chronic myelomonocytic leukemia (CMML) were randomized to 1 of 3 decitabine schedules: (1) 20 mg/m^2 intravenously daily for 5 days; (2) 20 mg/m^2 subcutaneously daily for 5 days; and (3) 10 mg/m^2 intravenously daily for 10 days. Randomization followed a Bayesian adaptive design. Ninety-five patients were treated (77 with MDS, and 18 with CMML). Overall, 32 patients (34%) achieved a complete response (CR), and 69 (73%) had an objective response by the new modified International Working Group criteria. The 5-day intravenous schedule, which had the highest dose-intensity, was selected as optimal; the CR rate in that arm was 39%, compared with 21% in the 5-day subcutaneous arm and 24% in the 10-day intravenous arm (P < .05). The high dose-intensity arm was also superior at inducing hypomethylation at day 5 and at activating P15 expression at days 12 or 28 after therapy. We concluded that a low-dose, dose-intensity schedule of decitabine optimizes epigenetic modulation and clinical responses in MDS.[44]

2.3 Studies of Decitabine in AML

We have previously investigated whether treatment with hypomethylating agents (5-azacytidine /decitabine) leads to an improved outcome in patients with AML who are older and/or have adverse cytogenetics.[45] Between January 2004 and December 2007, 81 patients (37 [46%] with AML [>or=20% blasts]; 44 [54%] with high-risk MDS) with chromosome 5 and 7 abnormalities were treated with hypomethylating agents as their initial therapy. These included 68 patients with complex (>or=3) abnormalities and 13 with <3 aberrations. During the same period, 151 patients (126 with AML, 25 with MDS) with chromosome 5 and 7 abnormalities (128 complex, 23 noncomplex) were treated with intensive chemotherapy (including cytarabine-based regimens in 72% and other regimes in 28%). The median ages for the 2 groups were 66 years and 61 years, respectively (ranges, 37-85 years and 19-89 years). Thirty-three (41%) patients in the hypomethylating group achieved complete remission (CR)

versus 53 (35%) in the chemotherapy group (P=.395). With a median follow-up of 51 weeks (range, 12-101 weeks) and 40 weeks (range, 5-128 weeks), 22 of 33 patients in the hypomethylating group and 33 of 53 patients in the chemotherapy group had developed disease recurrence. The median CR duration was 45 weeks and 23 weeks, respectively (P=.153). The overall survival was superior for the hypomethylating group compared with the chemotherapy group (P=.019). The investigators concluded that treatment with hypomethylating agents may be superior to chemotherapy in patients with chromosome 5 and 7 abnormalities.[45]

In a multicenter, phase II study, patients older than 60 years who had AML (ie, > 20% bone marrow blasts) and no prior therapy for AML were treated with decitabine 20 mg/m^2 intravenously for 5 consecutive days of a 4-week cycle. Response was assessed by weekly CBC and bone marrow biopsy after cycle 2 and after each subsequent cycle.[46] Patients continued to receive decitabine until disease progression or an unacceptable adverse event occurred. Fifty-five patients (mean age, 74 years) were enrolled and were treated with a median of three cycles (range, one to 25 cycles) of decitabine. The expert-reviewed overall response rate was 25% (complete response rate, 24%). The response rate was consistent across subgroups, including in patients with poor-risk cytogenetics and in those with a history of myelodysplastic syndrome. The overall median survival was 7.7 months, and the 30-day mortality rate was 7%. The most common toxicities were myelosuppression, febrile neutropenia, and fatigue. The investigators concluded that decitabine given in a low-dose, 5-day regimen has activity as upfront therapy in older patients with AML, and it has acceptable toxicity and a low 30-day mortality.[46]

In another phase II clinical trial with decitabine in older patients (>or=60 years) with previously untreated AML who were not candidates for or who refused intensive chemotherapy, subjects received low-dose decitabine at 20 mg/m^2 i.v. over 1 h on days 1 to 10. Fifty-three patients were enrolled with a median age of 74 years (range, 60-85).[47] Nineteen (36%) had antecedent hematologic disorder or therapy-related AML; 16 had complex karyotypes (>or=3 abnormalities). The complete remission rate was 47% (n = 25), achieved after a median of three cycles of therapy. Nine additional subjects had no morphologic evidence of disease with incomplete count recovery, for an overall response rate of 64% (n = 34). Complete remission was achieved in 52% of subjects presenting with normal karyotype and in 50% of those with complex karyotypes. Median overall and disease-free survival durations were 55 and 46 weeks, respectively. Death within 30 days of initiation of treatment occurred in one subject (2%), death within 8 weeks in 15% of subjects. They also examined the relationship of clinical response and pretreatment level of miR-29b, previously shown to target DNA methyltransferases. Higher levels of miR-29b were associated with clinical response (P = 0.02). They concluded that this schedule of decitabine was highly active and well tolerated in this poor-risk cohort of older AML patients and suggested that levels of miR-29b should be validated as a predictive factor for stratification of older AML patients to decitabine treatment.[47]

Most recently a multicenter, randomized, open-label, phase III trial compared the efficacy and safety of decitabine with treatment choice (TC) in older patients with newly diagnosed acute myeloid leukemia (AML) and poor- or intermediate-risk cytogenetics.[48]

Patients (N = 485) age ≥ 65 years were randomly assigned 1:1 to receive decitabine 20 mg/m^2 per day as a 1-hour intravenous infusion for five consecutive days every 4 weeks or TC (supportive care or cytarabine 20 mg/m^2 per day as a subcutaneous injection for 10 consecutive days every 4 weeks). The primary end point was overall survival (OS); the secondary end point was the complete remission (CR) rate plus the CR rate without platelet recovery (CRp). Adverse events (AEs) were recorded. The primary analysis with 396 deaths (81.6%) showed a nonsignificant increase in median OS with decitabine (7.7 months; 95% CI, 6.2 to 9.2) versus TC (5.0 months; 95% CI, 4.3 to 6.3; P = .108; hazard ratio [HR], 0.85; 95% CI, 0.69 to 1.04). An unplanned analysis with 446 deaths (92%) indicated the same median OS (HR, 0.82; 95% CI, 0.68 to 0.99; nominal P = .037). The CR rate plus CRp was 17.8% with decitabine versus 7.8% with TC (odds ratio, 2.5; 95% CI, 1.4 to 4.8; P = .001). Adverse events were similar for decitabine and cytarabine, although patients received a median of four cycles of decitabine versus two cycles of TC. The most common drug-related AEs with decitabine were thrombocytopenia (27%) and neutropenia (24%). Therefore, in older patients with AML, decitabine improved response rates compared with standard therapies without major differences in safety. An unplanned survival analysis showed a benefit for decitabine, which was not observed at the time of the primary analysis.[48]

2.4 Study Rationale

The current standard dose for decitabine in MDS and AML is 20 mg/m^2 daily x 5 days. A study conducted by the Ohio State University has produced significantly higher response rate when an extended schedule of decitabine 20 mg/m^2 daily x 10 days was used. The objective of the study is to demonstrate whether this extended 10 day schedule is superior compared to the standard 5 days schedule in producing responses and at least comparable with regards to toxicity and response duration.

3.0 Decitabine

3.1 Chemical Name

- 4-amino-1-(2-deoxy-ß-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H)-one
- Other Names: deoxyazacytidine, 5-aza-2'-deoxycytidine, 1-(2'-deoxy-D-ribofuranosyl)-5-azacytosine, DAC, 5-Aza-CdR, dezocitidine
- Classification: Antimetabolite, DNA hypomethylating agent
- Molecular Formula: C8H12N4O4 M.W.: 228.21
- Approximate Solubility: Slightly soluble in water (25mg/mL), ethanol and virtually insoluble in chloroform

3.2 Mode of Action: Hypomethylation of DNA followed by stimulation of gene expression and cell differentiation. Inhibits DNA methyltransferase. Targets for latter mechanism are selected genes silenced by DNA methylation. Inhibits DNA synthesis

3.3 How Supplied: Decitabine is supplied as a lyophilized white to almost white, finely crystalline, odorless powder for injection in 20mL vial glass vials, containing 50 mg of decitabine, monobasic potassium phosphate, and sodium hydroxide.

3.4 Preparation: When reconstituted with 10 mL of sterile water for injection each mL will contain 5 mg of decitabine, 6.8 mg of KH2PO4, and approximately 1.1 mg NaOH. The pH of the resulting solution is 6.5 - 7.5.

The reconstituted solution can be further diluted to a concentration of 1 mg/mL or 0.1 mg/mL in cold infusion fluids (0.9% Sodium Chloride Injection USP, 5% Dextrose in Water Injection, USP, or Lactated Ringer's Injection USP).

3.5 Storage and Stability: Vials should be stored at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). After reconstitution, use decitabine within 15 minutes. If diluted with cold (2°C to 8°C) fluids, the diluted solution is stable for up to 7 hours if stored between 2°C and 8°C (36°F to 46°F).

Reconstitution and dilution of the powder for injection (with 10mL of sterile water for injection) results in a rapidly decomposing solution. The concentration of decitabine in the reconstituted and diluted solution decreases about 10 % after 4 hours at 25°C or about 10% after 24 hours at 4°C. Since 10% is the maximum allowable decomposition, and the solution will also decompose during administration (infusion), the solution should be prepared just prior to administration. If this is not possible the solution should be prepared at least twice a day and kept in a refrigerator (2-8°C) until administration. Furthermore, the solution should be prepared only with cold infusion fluids at a temperature of 2-8°C; (36-46°F). This solution can be infused over a maximum period of 3 hours.

3.6 Route of Administration: Intravenous

3.7 Precautions

Drug handling precautions will be strictly followed. Skin contact with the solution should be avoided and protective gloves should be worn. Drug spilling can be inactivated by 2 M sodium hydroxide solution. The skin should be treated with a borax buffer solution pH 10 and after that thoroughly washed with water and soap.

3.8 Drug supply and distribution: Decitabine will be obtained from commercial source.

3.8 Reported Adverse Events and Potential Risks

Autoimmune reaction and/or chemical imbalances in the blood (antiplatelet antibodies, erythema nodosum), decreased hemoglobin, decreased leukocytes (total WBC), decreased neutrophils/granulocytes (ANC/AGC), decreased platelets, drowsiness, fatigue (lethargy, malaise, asthenia), alopecia, diarrhea, peritonitis, nausea, vomiting, anorexia, stomatitis/pharyngitis (oral/pharyngeal, mucositis), increase bilirubin, increased SGOT (AST) (serum glutamic oxaloacetic transaminase), increased SGPT (ALT) (serum glutamic pyruvic transaminase), liver damage, depressed level of consciousness, abdominal pain or cramping.

Also reported on Decitabine trials but with the relationship to Decitabine still undetermined: allergic reaction; allergic rhinitis ; GVHD; bone marrow cellularity; atrial fibrillation; cardiac ischemia/infarction; cardiopulmonary arrest; hypotension; left ventricular systolic dysfunction; right ventricular dysfunction; fever; insomnia; rigors/chills; weight loss; pruritus; rash; anal ulcer; ascites; constipation; distension; esophagitis; GI obstruction; ileus; taste alteration; CNS hemorrhage; GI hemorrhage; lung hemorrhage; nose hemorrhage; petechiae; urinary hemorrhage; cholecystitis; liver dysfunction/failure; infection without neutropenia; opportunistic infection; perianal abscess; head/neck edema; alkaline phosphatase; creatinine; hypercalcemia; hyponatremia; fracture; agitation; CNS ischemia; confusion; depression; dizziness; extrapyramidal/involuntary movement; motor neuropathy; psychosis; seizure; sensory neuropathy; bone pain; headache; muscle pain; urinary pain; ARDS; bronchospasm; cough; dyspnea; hiccoughs; pneumonitis/pulmonary infiltrates; cystitis; renal failure; urinary frequency; phlebitis; thrombosis/thrombus/embolism; veno-occlusive disease.

4.0 Eligibility Criteria

4.1 Inclusion criteria

1. Patients with previously untreated AML (by the WHO criteria, i.e.$\geq$ 20% blasts) Prior biologic therapies (such as growth factors) and targeted therapies administered for the treatment of prior myelodysplastic syndrome are allowed, with the exception of hypomethylating agents 5-azacytidine or decitabine. Patients must have been off such therapy for 1 week prior to entering this study and recovered from the toxic effects of that therapy, unless there is evidence of rapidly progressive disease. Hydroxyurea, and a single dose of cytarabine up to 3 g/m^2, is permitted for control of counts prior to treatment.

2. Patients $\geq$ 60 are eligible if not a candidate for standard cytarabine plus anthracycline chemotherapy as determined by Kantarjian's score (Appendix D) Patients younger than 60 may also be included if felt not to be a candidate for intensive anthracycline plus cytarabine based chemotherapy.

3. Performance 0-3 (ECOG).

4. Adequate liver function (Total bilirubin of < 2 mg/dl) unless due to hemolysis, leukemia organ infiltration or Gilbert's syndrome and renal function (creatinine < 2.5 mg/dl).

5. Signed informed consent

4.2 Exclusion Criteria

1. Nursing and pregnant females. Female patients of childbearing potential and male patients should practice effective methods of contraception such as double barrier method. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Negative urine pregnancy test (women of childbearing potential)

2. Active and uncontrolled infections.

3. Uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, active significant other cancers requiring chemotherapy and/or radiation therapy within past 6 months (excluding non-melanoma skin cancer) or psychiatric illness/social situations that would limit compliance with study requirements.

5.0 Therapy

5.1 Protocol registration:

All patients will be registered through the Protocol Data Management System (PDMS) or Clinical Oncology Research (CORe). The study chairman must be notified before any patients are registered.

5.2 Therapy

The initial Decitabine courses should be given at MD Anderson Cancer Center (or Regional Cancer Centers), either as in-patient or as out-patient. Decitabine will be dosed based on actual body weight. However, at the treating physician's discretion an adjusted body weight may be used, if actual body weight is >40% over ideal body weight. Adjusted body weight = ((Actual body weight – Ideal body weight) 0.4) + Ideal body weight. Decitabine may be subsequently administered at any of the Regional Cancer Centers (RCC) or at the patients' local oncologist offices. Patients will be assigned in a selective Bayesian design to one of two treatment arms.

1) Decitabine 20 mg/m^2 IV over approximately 1 hour daily x 5.
2) Decitabine 20 mg/m^2 IV over approximately 1 hour daily x 10.

Start at dose level 0 as shown below. There will be no dose escalation of Decitabine.

Patients will receive one course every 4-8 weeks; however delays of more than 8 weeks may be allowed if determined by the investigator to be in the best interest of the patient after discussion with the PI and the discussion documented in the patient's medical record. Doses may be given as close as 8 hours apart if approved by the PI. If prolonged aplasia and/or a major infection is seen, may decrease 1 dose level after marrow regeneration. This will be rounded to the following daily lower dose (15, 10, 7.5, 5 mg/m^2). Otherwise continue at the same dose level every 4-8 weeks depending on recovery of counts and disease status. If persistent disease, start next course regardless of counts. If marrow complete response is achieved with adequate cellularity, start subsequent courses when granulocytes $\geq$ 0.75 x 10^9/L and platelets $\geq$ 50 x 10^9/L.

Patients on the 10 day regimen will receive 5 day dosing after achieving CR during consolidation/maintenance. The 5 day dosing may also be initiated in these patients after the first course, if it is thought to be in the best interest of the patient after discussion with the PI and the discussion documented in the patient's medical record. Patients on the 5 day regimen will continue to receive the 5 day regimen after the first course whether in CR or not.

Modifications of dose schedule or dose level other than above are allowed if felt to be in the patients best interest to optimize response or reduce toxicity. These decisions should be documented in the patient's medical record.

	Decitabine mg/m^2 daily x days	
Dose level	10 days	5 days
0	20	20
-1	15	15
-2	10	10
-3	7.5	7.5
-4	5	5

5.3 Duration of Therapy

In the absence of treatment delays due to clinically significant study drug related adverse events, treatment may continue until one of the following criteria applies:

- Relapsed or clinically significant progressive disease; no response after $\geq$ 3 courses. Patients may continue therapy as long as they have stable disease, as long as it is determined to be in the best interest of the patient.

- Intercurrent illness that prevents further administration of treatment,

- Patient decides to withdraw from the study, or

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

- Up to a total of 24 courses of therapy; about 2 to 3 years.

a. Patients who show no evidence of life-threatening infection or hemorrhage, or less than grade 2 clinically significant drug related non-hematologic toxicity (vomiting included) may be given a subsequent course at the same dose as the previous course. If clinically significant drug-related grade 2 toxicity is observed, the patient must have recovered to grade $\leq$1 before institution of the next course. If patients have clinically significant drug-related grade 3-4 non-hematologic toxicity, they may receive a subsequent course at one reduced dose level after resolution of toxicity to $\leq$ grade 1.

b. Patients with a response (defined under section 10.0) and pre-course counts of granulocytes $>10^9$/L and platelets $>50 \times 10^9$/L who have sustained low counts of granulocytes $<0.5 \times 10^9$/L or a platelet count $<30 \times 10^9$/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.

c. Patients may receive further courses of therapy not earlier than 4 weeks from the previous course, provided the patient has recovered from myelosuppression considered to be related to chemotherapy. Patients with rapid increase in blasts may start additional therapy before 28 days from start of the previous course.

d. A minimum of 1 full course (or 4 weeks from the start of therapy) 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 decitabine will be considered evaluable for toxicity. Patients achieving a partial response or with stable disease may continue on therapy until definite evidence of disease progression.

e. Patients who achieve CR or CRi may be treated with additional courses of decitabine every 4-8 weeks or longer if approved by the PI. The dose of decitabine can be adjusted down by one dose level for toxicity (see above). Patients may be taken off study or treated on other programs (e.g. bone marrow transplantation) if this is judged more appropriate for their therapy and overall outcome. Delays of more than 8 weeks to subsequent course may be allowed if determined by the investigatory to be in the best interests of the patient after discussion with the PI and the discussion documented in the patient’s medical record. Abnormalities in chemistries including BUN, LDH (lactic dehydrogenase), alkaline phosphatase, magnesium, glucose, phosphorus, calcium and electrolytes will not be reported as adverse events. These are indications of disease process and not of toxicity, therefore clinically insignificant. Delayed recovery in patients post CR is common in MDS and AML not associated with significant clinical complications such as bleeding or infections. Therefore, delayed myelosuppression after a course in CR will be

reported as ADR only if platelets < 20 x 10^9/L or granulocytes < 0.5 x 10^9/L 6 weeks after completion of treatment.

5.4 Concomitant medications

GCSF or other growth factors are permitted on therapy only as per ASCO guidelines. Use of hydroxyurea with rapidly proliferative disease is allowed for the first two weeks on therapy.

6.0 Correlative studies

One of the goals of this protocol is to measure the biological effect of epigenetic therapies. Several correlative laboratory studies will be carried an attempt to assay the biological effect of decitabine. Patient samples including peripheral blood and bone marrow will be collected prior to, during and after completion of treatment.

6.1 Gene-mutations effecting methylation machinery

Mutations of several genes believed to be important in the methylation apparatus of the cell have been recently described in patients with acute myeloid leukemia (AML) but their presence has not been correlated with a worse or better outcome using hypomethylating agents. We have previously evaluated the association of mutations in *IDH1, IDH2, DNMT3A*, and *EZH2* with the outcome [complete response (CR) rate, event free survival (EFS) and overall survival (OS)] among patients older than 60 with AML (≥ 20% blasts) treated with hypomethylating agents as their first line of treatment. *TET2* mutations were not evaluated due to lack of available material. Among the 68 patients (median age 72 years; range, 60 – 83) with available data, 11 patients (16%) had *IDH1* or *IDH2* mutations (mutually exclusive) and 10 patients (15%) had *DNMT3A* mutations with 5 patients (7%) having both *IDH* and *DNMT3A* mutations. Cytogenetics was diploid in 19 (28%), abnormal chromosome 5/7 and/or complex in 27 (40%), trisomy 8 in 5 (7%), miscellaneous in 14 (21%), and insufficient in 3 (4%). Presence of *IDH* mutations was associated with a diploid karyotype and the presence of *NPM1* mutations (p=.03 and p=.02, respectively) but not with *FLT3*-ITD or *RAS* mutations (present in 7 and 4 patients, respectively). *DNMT3A* mutations were not associated with any specific karyotype or with the presence of *NPM1*, *FLT3*-ITD, or *RAS* mutations. None of the 68 patients had *EZH2* mutations. All patients were treated with hypomethylating agents [decitabine in 39 (57%) and 5-azacytidine in 29 (43%)] with 42 patients (62%) receiving concomitant histone deacetylase inhibitor therapy (SAHA or valproic acid). Overall, 17 patients (25%) achieved CR; the presence of *IDH* or *DNMT3A* mutations or both was not associated with achievement of CR. With a median duration of follow-up of 60 months, the median EFS is 3.3 months (range, 0.25 – 3.75 months) and the median overall survival is 6 months (range, 0.25 – 90.5 months). Presence of *IDH* mutations was not associated with an impact on EFS (p=.29) or OS (p=.14). Similarly, *DNMT3A* mutations were not associated with an effect on EFS (p=.21) or OS (p=.58). The presence of both *IDH* and *DNMT3A* mutations was also not associated with a better or worse response, EFS, or OS as compared with patients with neither mutation. We

were unable to detect an association between presence of *IDH1/2* and *DNMT3A* mutations and outcome in this elderly population of patients with AML treated with epigenetic modulators.

In this study we propose to evaluate the potential effect of mutations of 1DH1, IDH2, DNMT3A, TET2, EZH2 and other genes believed to be in the DNA methylation program prospectively by assessing the patients for the presence of these mutations prior to the initiation of therapy. We will also examine follow-up samples to determine if the leukemic blasts harboring these genes remain after therapy.

Using more sophisticated assessments of gene panels and whole exome sequencing we will expand this prospective evaluation and will determine if certain gene signatures are associated with response.

We will also evaluate the levels of 2-hydroxyglutarate in patients with DNMT3A mutation and will determine if there is any association with response to therapy and outcome.

6.2 Gene specific methylation.

The proposed mechanism of action of hypomethylating agents such as decitabine is through their action against DNA methyltransferase enzymes responsible for methylation of promotor regions (and hence silencing of) genes responsible for differentiation. We will examine this hypothesis though the examination of gene-specific and global methylation of the leukemic cell genome pre and post therapy. Quantitative methylation analysis of the p15 promoter and other genes, including Alu elements will be performed using "COBRA" analysis (Combined Bisulfite and Restriction Analysis). This technique takes advantage of the ability of bisulfite to selectively convert cytosine to uracil, but without affecting 5-methylcytosine. Thus DNA can be treated with bisulfite, amplified by PCR, and the PCR product digested with restriction enzymes which will quantitatively assess gene specific methylation depending on the converted DNA sequence.

6.2.1 Assessment of genome wide methylation. Total genome DNA methylation can be measured using high pressure liquid chromatography and assaying directly for 5-methylcytosine. This technique however requires large amounts of DNA sample (approximately 50ug).

Therefore COBRA analysis described above will be used to assess methylation of genes and Alu repetitive elements in the patient samples. Alu elements are short DNA repeats which litter the genome. It is estimated that there are one million Alu's in the human genome. These elements are also heavily methylated, and therefore allow the opportunity to simultaneously study demethylation of one million sites in the genome rather than a single gene.

6.3 Micro-RNA analysis

As mentioned previously, higher levels of miR-29b were associated with clinical response. We will evaluate this prospectively by analyzing pre-treatment bone marrow samples for the relevant micro-RNAs in collaboration with the laboratory of Dr. George Calin.

6.4 Assessment of immune function and effector cells

The role of an intact immune effector cell function towards response and eradication of minimal residual disease will be evaluated in collaboration with Dr. Katy Rezvani's laboratory. Immune suppression is an unavoidable consequence of current therapeutic modalities and is associated with significant morbidity and mortality due to opportunistic infections. We have developed sensitive laboratory methods that allow the study of immune subset recovery (T cell, B cell and NK cell recovery). At this time, careful studies of immune recovery are not routinely performed in subjects with AML undergoing treatment with decitabine. Such studies would allow us to better understand the effects of the drug on the recovery of immune function in AML[50-53].

We propose to systematically collect specimens of blood, bone marrow and plasma from patients with AML undergoing therapy with decitabine. Samples may be collected prior to initiation of therapy, at the time of achieving complete remission and suspected relapse and prior to every 3rd or 4th cycle of therapy; Omissions will not be considered as violations. Assays to be performed may include the following: 1) T cell immunophenotyping (to define the recovery of T cell subsets associated with a "naïve" or "memory" T cell phenotype); 2) Functional studies of antigen-specific T cell responses to pathogens and to leukemia-associated antigens (e.g., to defined epitopes that have been identified as potential malignancy-specific targets);[1,2,3] 3) Studies examining the molecular diversity of the T cell repertoire;[5] 4) Assays of plasma levels of cytokines (e.g., interleukin-7) that may be important in T cell reconstitution; 5) Studies to assess the role of other immune subsets involved in anti-pathogen and anti-leukemia responses including natural killer (NK) cells, B lymphocytes, antigen presenting cells (e.g. dendritic cell subsets) and subsets involved in immunoregulatory pathways such as macrophages and myeloid derived suppressor cells; 6) Molecular studies will be performed on total RNA or DNA extracted from PBMC to assess the role of immune-related genes in treatment outcome (infection and relapse). Some or all of these studies may be performed at the time of collection. In most cases, cryopreserved PBMC or plasma samples will be used for these assays. Cryopreserved cells in excess of requirements for studies of immune reconstitution will be banked to allow access of other investigators to these specimens.

7.0 Pre-study evaluations (to be performed within 14 days unless otherwise noted)

7.1 History and physical exam.

7.2 CBC, platelet count, differential; creatinine, bilirubin, SGPT (within 7 days).

7.3 Bone marrow aspirate (within 14 days) and cytogenetics

7.4 Molecular studies in bone marrow specimens for FLT3-ITD, FLT3-TKD, NPM1, IDH1, IDH2, DNMT3A, TET2, EZH2, RAS, JAK2 (if appropriate, e.g. in patients

with history of MPN), and the Leukemia mutation panel (whichever is available at the time of enrollment)

7.5 Whole genome sequencing (performed at Dr. Lynda Chin's laboratory)

7.6 Blood sample (20-50 cc) and marrow aspirate sample (2 cc) for methylation studies, to be sent to Dr. Garcia-Manero's laboratory. Not all samples may be collected at all times points and omissions will not be considered deviations. Samples obtained prior to consenting may be used.

7.7 Blood sample (25-35cc) will be collected for assessment of immune function and effector cells to be sent to Dr. Katy Rezvani's lab. Not all samples may be collected at all times points and omissions will not be considered deviations. Samples obtained prior to consenting may be used.

7.8 Pregnancy (urine or blood) test only for women of childbearing potential within 7 days.

8.0 Evaluations during the study

8.1 CBC, differential, platelet count 1-2 times weekly for 1st course, then every 2-4 weeks while on therapy. The frequency can be reduced to once per cycle after achieving CR or after sixth cycle (whichever first)

8.2 Creatinine, bilirubin, SGPT weekly for 1st course, then every 2-4 weeks while on therapy. The frequency can be reduced to once per cycle after achieving CR or after sixth cycle (whichever first)

8.3 Bone marrow aspiration to document remission then every 1-3 courses.

8.4 Cytogenetics at remission if abnormal pretherapy any time a bone marrow exam is performed. Omissions will not be considered as deviations.

8.5 Molecular testing depending on the positive tests pre-study (specific mutations, whole genome sequencing) any time a bone marrow exam is performed. Omissions will not be considered as deviations.

8.6 Multi-parameter flow cytometry for the presence of leukemia associated immunophenotype (minimal residual disease) any time a bone marrow exam is performed. Omissions will not be considered as deviations..

8.7 Blood samples (20-50cc) on days 1 (any time prior to first dose), 5 and 10 (+/- 3 days) of therapy (optional); marrow sample (2 cc) every 1-3 courses (optional). Samples are to be sent to Dr. Garcia-Manero's lab (pager 713-404-0277). (Not all research samples may be collected at all time-points and omissions will not

be considered deviations.) Precourse samples may be obtained after day 27 of the preceding course, but prior to first dose of next course.

8.8 Blood sample (25-35cc) will be collected for assessment of immune function and effector cells at the time of remission, relapse, and about every 3 – 4 cycles. Samples are to be sent to Dr. Katy Rezvani's lab. (Not all samples may be collected at all times points and omissions will not be considered deviations.)

8.9 Peripheral blood specimens will be obtained at the time of complete remission and relapse, and evaluated for the presence of various T-cell subsets, NK cells and other immune effector measures

9.0 Response criteria

9.1 Complete Remission: Normalization of the peripheral blood and bone marrow with $\leq$5% bone marrow blasts, a peripheral blood granulocyte count $\geq$ (1.0 x 10^9/L, and a platelet count $\geq$ 100 x 10^9/L).

9.2 Partial Remission: as above except for the presence of 6-15% marrow blasts, or 50% reduction if <15% at start of treatment.

9.3 Complete remission with incomplete recovery (CRi): meets all criteria for CR except for platelet recovery to $\geq$100 x 10^9/L and/or granulocyte count $\geq$ (1.0 x 10^9/L

9.4 Clinical benefit: platelets increase by 50% and to above 30 x 10^9/L untransfused (if lower than that pretherapy); or granulocytes increase by 100% and to above 10^9/L (if lower than that pretherapy); or hemoglobin increase by 2 g/dl; or transfusion independent; or splenomegaly reduction by $\geq$ 50%; or monocytosis reduction by $\geq$ 50% if pretreatment > 5 x 10^9/L.

10.0 Criteria for removal from the study

10.1 No improvement or clinically significant progressive disease

10.2 Patient refusal

10.3 Pattern of patient non-compliance; physician judgment

11.0 Statistical Considerations

11.1 General description

This is a Phase II open-label, efficacy and toxicity study of two treatment arms (arm A: Decitabine 20mg/m^2 IV daily for 5 days versus arm B: Decitabine 20mg/m^2 IV daily for 10 days) in subjects with AML $\geq$ 60 or unfit for standard cytotoxic chemotherapy. Patients will be enrolled to evaluate the 2 dose schedules. For both arms, one course will consist of 4 weeks. An adaptive randomization design will be employed to compare the efficacy between the two arms. The primary efficacy outcome is the complete response which is defined as the complete remission (CR) or complete remission with incomplete recovery (CRi) assessed after three cycles. At the end of the trial, we will estimate the probability that one arm is superior to the other. We will also evaluate toxicity on each arm. A maximum of 100 patients will be accrued, at an expected accrual rate of 5 patients per month.

11.2 Randomization

The Department of Biostatistics will provide and maintain a website ("Clinical Trial Conduct": https://biostatistics.mdanderson.org/ClinicalTrialConduct/) for patients enrolled on this study. The Clinical Trial Conduct website resides on a secure server, and access is gained through usernames and passwords provided to personnel responsible for enrolling patients and updating patient data. The website is accessed through a browser using secure socket layer (SSL) technology. Personnel responsible for enrolling patients on trials, which includes the principal investigator(s), research nurse(s), and data coordinator(s), will be trained by members of the Department of Biostatistics (specifically the statistical collaborators on the study) in the use of the trial website; the importance of timely updating of follow-up times and recording of events will be emphasized in training.

11.3 Study Design

A maximum of 100 patients will be adaptively randomized into two treatment groups:

Arm A: Decitabine 20mg/m2 IV daily for 5 days

Arm B: Decitabine 20mg/m2 IV daily for 10 days

Patients will be assigned to receive different schedules of Decitabine, using an adaptive procedure that bases assignment probabilities on observed results in preceding patients. At first, 20 patients will be assigned to each arm with equal probability. As efficacy data accrues, patient assignment to the two arms will become unbalanced in favor of the one that has the higher complete response rate (RR). The RR for both arms will be estimated with a 95% CI (similar to Bayesian highest posterior density interval).[49]

Based on previous studies, we expect a RR of about 30% in both arms. Therefore, we assume RR has a prior Beta distribution (0.6, 1.4) with mean 0.3. Let RRa and RRb denote the response rates for arm A and arm B, respectively. Beginning with the 21st patient in each arm and for each subsequent patient, we will compare RRa with RRb, incorporating data from all patients with evaluable response. In order to avoid favoring one arm earlier in a large trial, instead of assigning patients with posterior probability (Pa=Pr(RRa>RRb|data) and Pb=1-Pa), we use the following formula to assign patients:

Aa=$\frac{\sqrt{Pa}}{\sqrt{Pa}+\sqrt{Pb}}$, Ab=1-Aa, where Aa is the probability of assigning patients to arm A, Ab is the probability of assigning patients to arm B, Pa is the posterior probability that arm A is superior to arm B and Pb is the posterior probability that arm B is superior to arm A.

If at any point during the trial Pr(RRa>RRb|data)>0.95(or <0.05) the schedule A(or B) will be selected as superior. If accruing information gives strong evidence that a RR of 30% or greater is unlikely to be true for any one of the treatment arms (Pr(RRa or RRb>0.3|data)<0.05), assignment to that arm will be stopped. If the maximum of 100 patients is enrolled and evaluated and Pr(RRa>RRb|data)>0.9 (or <0.1), we will declare that arm A(or B) has a higher RR rate than arm B (or A). Otherwise, the trial will be inconclusive. We used simulation (5,000 simulations per scenario) to evaluate the performance of the adaptive randomization procedure under several different scenarios (Table 2). Adaptive Randomization version 4.1 has been used for the simulation. Adaptive Randomization version 4.1 has been used for the simulation.

11.4 Toxicity Monitoring

Evidence of Toxicity will be monitored closely in all patients. For each arm, treatment will be terminated if Pr(non hematological grade 3 or higher clinically significant study drug related Toxicity >0.3 | data) >0.95. We assume P_E ~ beta (0.3, 0.7). Table 1 shows the simulation of the trial. The treatment will be stopped accrual if the number of patients with clinically significant study drug related toxicities equal to or greater than indicated (i.e., #pts with tox) among the number of patients accrued (i.e., #pts): 2/2, 3/3, 4/5, 5/7, 6/9, 7/11, 8/14, 9/16, 10/19, 11/21, 12/24, 13/27, 14/29, 15/32, 16/35, 17/38, 18/40, 19/43, 20/46, 21/49, 22/52, 23/54, 24/57, 25/60, 26/63, 27/66, 28/69, 29/72, 30/75, 31/77. Multc99 version2.1 has been used for the simulation and boundary computation.

Table 1: Operating characteristics of Safety Monitoring (based on 10000 simulations)

True probability	Stop probability	median sample size (interquantile)
0.2	0.06	80 (80, 80)
0.25	0.13	80 (80, 80)
0.3	0.28	80 (45, 80)
0.35	0.53	65 (12, 80)

Table 2: Operating characteristics of adaptive randomization design to compare DAC in two schedules. Randomly assign 20 patients to each arm with equal probability before adapting the randomization

	Arm A	Arm B
RR Rate	0.05	0.1
Expected # of Patients	20.1	20.1
Pr(Select)	0.02	0.14
Pr(Select Early)	0.01	0.10
Pr(Stop Early)	0.95	0.73
RR Rate	0.1	0.3
Expected # of Patients	21.5	22.6
Pr(Select)	0.003	0.66
Pr(Select Early)	0.001	0.52
Pr(Stop Early)	0.96	0.07
RR Rate	0.3	0.65
Expected # of Patients	21.7	24
Pr(Select)	0.0002	0.99
Pr(Select Early)	0.0002	0.98
Pr(Stop Early)	0.98	0.0002
RR Rate	0.3	0.3
Expected # of Patients	40.2	40.3
Pr(Select)	0.15	0.15
Pr(Select Early)	0.11	0.12
Pr(Stop Early)	0.21	0.2
RR Rate	0.5	0.6
Expected # of Patients	36.7	43
Pr(Select)	0.03	0.47
Pr(Select Early)	0.03	0.42
Pr(Stop Early)	0.42	0.03

11.5 Analysis Plan

Data analysis will be performed using SAS or S-plus, as appropriate. All patients will be included in the intent-to-treat analysis for efficacy. Demographic and disease characteristics of the patients at registration will be summarized using descriptive statistics such as mean, standard deviation (SD), median and range. The RR for both arms will be estimated by Bayesian posterior estimates, along with the 95% credible intervals. The posterior probability that one treatment is better than the other will be computed, and the 95% credible interval of the posterior probability will also be estimated. The time-to-event variables will be estimated by Kaplan-Meier method. The survival difference between the two treatment schedules will be compared by Log-rank test. The OS is defined as the time period from the start of treatment till death or last follow-up whichever comes first. Duration of response (DoR) is for the patients who achieve CR or CRi, and it is defined as the time between the date of response confirmed (CR or CRi) and the date of progression or death (whichever occurs first). DoR will be censored on the date of last follow-up.

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